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Document for Epichlorohydrin
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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on epichlorohydrin. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of epichlorohydrin
- Toxicokinetics and human exposure to epichlorohydrin
- Health Effects of epichlorohydrin in humans and animals
- Mechanisms of toxicity of epichlorohydrin
- Quantification of toxicological effects of epichlorohydrin

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Prepared for

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LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
ADI	Acceptable daily intake
bw	Body weight
DNA	Deoxyribonucleic acid
FSH	Follicle-stimulating hormone
GC	Gas chromatography
GLC	Gas-liquid chromatography
HA	Health Advisory
i.p.	Intraperitoneal
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LH	Luteinizing hormone
LOAEL	Lowest-observed-adverse-effect level
LT_{50}	Time of exposure lethal to 50% of recipients
MITI	Ministry of International Trade and Industry (Japan)
MS	Mass Spectrometry
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PHA	Phytohemagglutinin
RNA	Ribonucleic acid
s.c.	Subcutaneous
SCE	Sister-chromatid exchange
SMR	Standardized mortality ratio
SNARL	Suggested-no-adverse-response level
STEL	Short-term exposure limit
TEPA	Tris (1-aziridinyl)-phosphine oxide
TLC	Thin layer chromatography
UF	Uncertainty factor

I. EXECUTIVE SUMMARY

Epichlorohydrin has a boiling point of 116.1°C, a vapor pressure of 12 torr at 20°C and an aqueous solubility of 66,000 ppm (by weight). Hydrolysis and volatilization are likely the dominant fate process, although volatilization and biodegradation may be important.

Epichlorohydrin is readily absorbed following oral, dermal or inhalation exposures. Following oral administration of radiolabeled epichlorohydrin, peak tissue levels of radioactivity occur in 2-8 hours. The highest tissue levels occur in the kidneys, liver, pancreas, spleen and adrenals. The nasal turbinates and lacrimal glands also accumulate relatively high levels of radioactivity following inhalation exposures. Epichlorohydrin is rapidly removed from blood and is not likely to accumulate during chronic exposure; however, the metabolites are more persistent and may have a slight potential for accumulation.

Epichlorohydrin has two electrophilic centers and may bind to cellular nucleophiles. It is also a substrate for epoxide hydratase, resulting in the formation of α-chlorohydrin. The α-chlorohydrin may be oxidized to oxalic acid, converted to glycidol, or be phosphorylated to yield 3-chloroglycerophosphate.

The metabolites of epichlorohydrin are excreted in the urine and expired air, with urinary excretion accounting for ~66% of the total by these routes. Only minor amounts are excreted in the feces.

Epichlorohydrin is acutely toxic following oral, percutaneous, subcutaneous or respiratory exposure, producing similar symptoms in each case. At the site of application, epichlorohydrin is a strong irritant. The major acute systemic effects occur in the central nervous system, with death being due to depression of the respiratory center. The major internal organs affected are the lungs, liver and kidneys.

The acute oral LD_{50} of epichlorohydrin is ~250 mg/kg bw in both rats and mice. Reported LC_{50}s for inhalation exposures range from 1362-4000 mg/m^3.

Dermal exposure results in discoloration and superficial desquamation within a few hours of exposure. Continued exposure produces a superficial necrosis. The skin becomes dry, parchment-like and brown. These effects are slowly reversible after exposure is discontinued.

The pathology produced by chronic exposure to epichlorohydrin is qualitatively similar to that observed following an acute exposure. The major target organs for epichlorohydrin are the nasal turbinates, lungs, kidneys, male reproductive organs and the central nervous system. Toxicity in the nasal tubinates and lungs appears to be due largely to the irritant activity of epichlorohydrin. Kidney damage tends to be localized in the convoluted tubules, though glomerular involvement has been reported in some cases. Two types of infertility are produced by oral exposure to epichlorohydrin. At high doses (50-100 mg/kg bw/day) a prolonged, possibly permanent, infertility is produced in males. At lower doses (10-20 mg/kg bw/day) a reversible infertility is produced.

Epichlorohydrin has been found to be carcinogenic in laboratory animals following both oral and inhalation exposures. Oral exposure produces a dose-dependent increase in forestomach tumor characterized as squamous and basal hyperplasia, squamous cell papillomas or squamous cell carcinomas. Inhalation exposure produces squamous cell carcinomas in the nasal cavities, many of which infiltrate and penetrate the bones of the skull. Epichlorohydrin does not appear to be a complete animal carcinogen following dermal exposure, but one study has indicated that it has weak initiating activity.

Epichlorohydrin has been found to be a potent inducer of base-pair substitution-type mutations in numerous prokaryotic systems. Incubation with mammalian liver homogenates results in a marked reduction in mutation frequency. Mutations have also been induced in eukaryotic cell cultures, but less consistent results have been obtained in vivo. Epidemiological studies have implicated epichlorohydrin in the production of DNA damage in lymphocytes of chemical plant workers. Sex-linked recessive mutations are induced in Drosophila when the compound is injected, but not when it is fed. Only negative results have been reported for the mouse dominant lethal assay and micronucleus assay.

In two seperate studies, no teratogenic effects have been detected that could be attributed to epichlorohydrin exposure. It has, however, been demonstrated to be a male antifertility agent in a number of reproduction studies.

In humans, acute effects have been reported following both dermal and inhalation exposures. Dermal exposure produces predominately local effects, but inhalation produces significant systemic effects. These systemic effects involve hepatic and renal toxicity and persist for at least 2 years after the exposure. Chronic exposure to epichlorohydrin has been associated with chromosome and chromatid breaks, decreased hemoglobin concentration, decreased erythrocyte counts and decreased leukocyte counts. Small, but not statistically significant, increases in the mortality due to lung cancer has been reported in workers exposed to epichlorohydrin. No effects on reproductive function have been detected.

Three mechanisms have been proposed by which epichlorohydrin may exert its toxicity. First, epichlorohydrin and at least two of its metabolites are electrophilic. Thus, these compounds are able to bind to many cellular nucleophiles, resulting in an alteration of cellular function. Second, one of the metabolites of epichlorohydrin, α-chlorohydrin-1-phosphate, is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase. The metabolite is believed to be involved in the antifertility effects of epichlorohydrin. The third mechanism of epichlorohydrin toxicity is thought to involve the metabolic production of oxalic acid. The metabolite precipitates as the calcium salt, producing the renal toxicity and possibly some of the antifertility effects of epichlorohydrin.

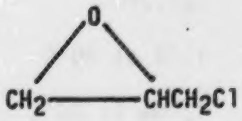
Based on the toxicity data found in the literature, 1-day, 10-day and lifetime adjusted acceptable daily intake (AADIs) have been calculated. The resulting 1-day HAs are 140 and 500 μg/ℓ drinking water for a 10 kg child and 70 kg adult, respectively. The corresponding 10-day HAs are 140 and 500 µg/ℓ drinking water. The lifetime AADI for noncarcinogenic effects is 76 µg/ℓ. However, epichlorohydrin is a demonstrated animal carcinogen, and under the assumptions used with the multistage carcinogenic risk assessment model, it is estimated that a water concentration of 3.54 µg epichlorohydrin/ℓ would result in a maximum lifetime cancer risk of $1x10^{-6}$. Using organoleptic data, thresholds of 0.5-1.0 mg/ℓ for odor perception and 0.1 mg/ℓ for irritating effects have been reported for epichlorohydrin.

II. GENERAL INFORMATION AND PROPERTIES

Structure and Identification

Epichlorohydrin is also known as 1-chloro-2,3-epoxypropane, 3-chloro-1,2-epoxypropane, (chloromethyl) oxirane, 2-(chloromethyl) oxirane and chloropropylene oxide.

It has the following structure:



The molecular formula is C_3H_5ClO.

The molecular mass is 92.53.

The Chemical Abstracts Service (CAS) registry number for this compound is 106-89-8, and the Registry of Toxic Effects of Chemical Substances (RTECS) number is TX4900000.

Chemical and Physical Properties

The physical properties of epichlorohydrin are summarized in Table II-1. The commercial product is a colorless liquid used in the manufacture of epoxide resins, surface active agents, pharmaceuticals and agricultural chemicals (Verschueren, 1983).

Stability in Water

The dominant removal process for epichlorohydrin in water is probably hydrolysis and volatilization, although biodegradation also may be significant.

TABLE II-1

Physical Properties of Epichlorohydrin

Property	Value	Reference
Boiling point	116.1°C	Verschueren, 1983
Melting point	-57.2°C	Riesser, 1979
Specific gravity	1.18 at 20°C	Verschueren, 1983
Vapor pressure	12 mm at 20°C	Verschueren, 1983
O/W Partition Coefficent	0.26*	
H_2O solubility	6.6 weight % at 20°C	Riesser, 1979
Lipid solubility	Soluble in most organic solvents	Riesser, 1979
Conversion factor	1 mg/m³ = 0.265 ppm 1 ppm = 3.78 mg/m³	Verschueren, 1983

*Calculated by the method of Hansch and Leo, 1979.

Mabey and Mill (1978), based on a review of the literature, reported the calculated hydrolysis half-life for epichlorohydrin to be 8.2 days at 20°C and pH 7. This is based on a hydrolysis rate constant of 9.8×10^{-7} sec^{-1} in neutral water. Epichlorohydrin in basic environmental waters will have a shorter half-life; acid waters will give the same half-life (Mabey and Mill, 1978).

Volatilization half-lives for epichlorohydrin cannot be calculated accurately based on the Whitman two film theory (Whitman, 1923) because of its solubility (virtually all environmental process models are based on this theory). However, Matsui et al. (1975) reported that volatilization accounted for most of the 89% loss over 24 hours of epichlorohydrin during an activated sludge fill and draw test. Aeration was at 5 ℓ/minute. In a similar experiment, Neeley (1976) aerated a 100 ppm solution of epichlorohydrin in a cylinder 39.4 cm deep and reported a rate constant of 2.32 cm/hr. Smith et al. (1979) reported the loss of epichlorohydrin in water from volatilization and hydrolysis. A screw-capped glass vial containing a 3% water solution of epichlorohydrin at room temperature lost 10% of the initial concentration in 7 hours; after 64.5 hours, 30% was lost. Based on their data, a half-life of 5.6 days would be expected for all processes. Since 8.2 days would be expected for hydrolysis alone, some volatilization was occurring (assuming the same temperature and pH). The volatilization rate of environmentally significant concentrations of epichlorohydrin in ambient waters cannot be estimated from these data, although volatilization is probably an important process.

Bridie et al. (1979) reported that 14% of theoretical BOD was consumed in 5 days at 20°C using an acclimated sewage sludge seed. The 5-day BOD using unacclimated seed was 3%; COD was 96% of theoretical. Based on this and the report of Sasaki (1978), who reported that epichlorohydrin was "significantly degraded" in a MITI test, it is anticipated that this compound may be significantly degraded in sewage treatment systems. Based on the above data, little can be said about the biodegradation of epichlorohydrin in environmental waters.

Summary

The physical properties of epichlorohydrin are summarized in Table II-1. The commercial product is a colorless liquid used in the manufacture of epoxide resins, surface active agents, pharmaceuticals and agricultural chemicals. Epichlorohydrin has a boiling point of 116.1°C, a vapor pressure of 12 torr at 20°C and a solubility of 66,000 ppm (by weight) (Verschueren, 1983; Riesser, 1979). Hydrolysis and volatilization are probably the dominant fate processes (Mabey and Mill, 1978), although biodegradation may be important (Bridie et al., 1979; Sasaki, 1978; Neeley, 1976).

III. TOXICOKINETICS

Absorption

Qualitatively, epichlorohydrin appears to be absorbed rapidly following either oral or inhalation exposures. Weigel et al. (1978) administered 10 mg [1,3-^{14}C] epichlorohydrin/kg bw in corn oil by gavage to groups of 21 male (250±54 g) and 21 female (208±24 g) CD rats. In the males, peak tissue levels of ^{14}C were observed at 2 hours after dosing. Groups of three males and three females were periodically killed as described in the Distribution Section. In the females, peaks of radioactivity in body tissues were observed at 2, 4 or 8 hours (see Distribution Section). Based on the excretion of ^{14}C (see Distribution Section), at least 96% of the administered dose was absorbed. Smith et al. (1979) have also reported the extensive absorption of epichlorohydrin in water in male (190-220 g) Fisher 344 rats following a single gavage exposure. Based on excretion data, the extent of absorption, ~100% within 72 hours after administration, appeared to be similar following doses of either 1 or 100 mg/kg bw.

Rossi et al. (1983a) administered one or seven daily oral doses of 50 or 200 mg epichlorohydrin in water/kg bw by gavage to male (25-30 g) Swiss mice. Blood epichlorohydrin concentrations were assayed by GC-MS. Following a single oral dose of 50 mg/kg bw, blood concentrations remained below the limits of detection (50 ng/mℓ); however, absorption was rapid following a dose of 200 mg/kg bw, resulting in peak blood concentrations of 500 ng/mℓ within 5 minutes and non-detectable blood levels within 20 minutes. Repeated dosing with seven consecutive doses of either 50 or 200 mg/kg/day did not appreciably alter the kinetics of epichlorohydrin or α-chlorohydrin, its major metabolite (see Distribution Section).

Smith et al. (1979) indicated that epichlorohydrin was nearly 100% absorbed following inhalation exposure. Following a 6-hour exposure of male (190-220 g) Fischer 344 rats to atmospheres containing 1 or 100 ppm epichlorohydrin (~3.78 or 378 mg/m^3), ~90% of the dose was excreted as metabolites in the urine and as $^{14}CO_2$ in the expired air within 72 hours. Uptake rates of 15.48 and 1394 μg/hr were calculated for exposures to 1 and 100 ppm, respectively. The investigators stated that these exposures correspond to doses of 0.37 and 33 mg/kg bw.

The toxicity study of Kremneva and Tolgskaya (1961) indicates that epichlorohydrin is also absorbed following dermal exposure. When the tails of mice were immersed in epichlorohydrin either for a single exposure of 1 hour, or for repeated exposures of 20-30 minutes/day on 2-3 successive days, toxic signs and death were observed within 3 days. The investigators did not specify what, if any, precautions were taken to avoid simultaneous exposure to epichlorohydrin vapors. The data are insufficient to determine the rate or extent of absorption.

Disbribution

The tissue distribution of epichlorohydrin has been investigated following both oral and inhalation administration. Weigel et al. (1978) administered a single dose of 10 mg [1,3-^{14}C]-epichlorohydrin/kg bw by gavage to male and female CD rats. Groups of three male and three female rats were sacrificed at 2, 4, 8, 12, 24, 48 and 72 hours post-dosing. In males, the peak tissue levels of ^{14}C were observed at 2-4 hours, with the highest levels occurring in the kidneys, liver, pancreas, spleen and adrenals. In females, the same tissues contained high levels of radioactivity; however, the time-course of distribution was considerably different. Peak levels of radioactivity occurred from 2-8 hours post-administration, depending on the tissue examined. Blood levels peaked at 2 hours, declined at 4 hours, and rose again at 8 hours, possibly indicating an initial distribution followed by metabolism and redistribution in female rats. These data are summarized in Table III-1 (male rats) and Table III-2 (female rats).

Smith et al. (1979) compared the distribution of epichlorohydrin in male Fisher 344 rats following oral (100 mg/kg bw) or inhalation exposure (100 ppm for 6 hours). Concentrations in the nasal turbinates were 4- to 5-fold greater than those in the lungs following inhalation, but not oral, exposure. At 3 hours post-exposure in the oral study or termination of inhalation exposure, the plasma levels of radioactivity were 36.1 and 18.3 mg/g, respectively. These data are summarized in Table III-3. The authors speculate that "this route dependent difference in distribution may be manifest as a route related difference in the toxicity of Epi."

Bioaccumulation and Retention

As discussed in the Excretion Section, the majority of epichlorohydrin administered to mice is rapidly metabolized and excreted through the urine and expired air. Blood levels of the parent compound peak at 5 minutes following a single oral dose and decline to the detection level (50 ng/mℓ) by ~25-30 minutes, indicating that unmetabolized epichlorohydrin should not accumulate in the body (Rossi et al., 1983a).

Following oral administration of $[^{14}C]$-epichlorohydrin to rats, blood and tissue levels of radioactivity remained elevated for considerably longer

TABLE III-1

Percent of Dose in Selected Tissues of Male Rats at Various Time Intervals Following an Oral Dose of 10 mg/kg ^{14}C-Epichlorohydrin[a,b]

Tissue	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours	48 Hours	72 Hours
Lungs	0.20±0.01	1.15±[c]	0.11±[c]	0.11±[c]	0.07±0.01	0.06±[c]	0.06±0.01
Liver	4.89±0.39	3.48±0.11	3.14±0.24	2.33±0.07	1.99±0.29	1.18±0.13	0.42±0.03
Kidneys	1.78±0.11	1.42±0.07	1.15±0.07	0.98±0.03	0.49±0.08	0.37±0.04	0.14±0.05
Testes	0.46±0.02	0.33±0.01	0.20±0.01	0.15±0.01	0.09±0.02	0.08±0.01	0.06±0.01
Blood	3.30±0.19	3.24±0.19	2.41±0.07	2.03±0.24	1.31±0.21	1.02±0.18	0.61±0.22
Urine	no sample	17.21±1.55	33.52±3.12	36.21±5.43	37.01±7.56	38.78±5.98	33.18±0.78
Fat	1.91±0.37	1.02±0.09	0.90±0.10	1.42±0.16	0.62±0.14	0.73±0.06	0.60±0.64
Stomach	11.48±1.39	17.47±2.74	2.10±1.50	0.40±0.06	0.29±0.07	0.18±0.01	<0.01

[a] Source: Adapted from Weigel et al., 1978

[b] Each value represents the mean ± S.E.M of duplicate determinations on three animals at each time interval.

[c] <0.01

TABLE III-2

Distribution of ^{14}C-Epichlorohydrin in Selected Tissues of Female Rats at Various Time Intervals Following a 10 mg/kg Oral Dose[a,b]

Tissue	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours	48 Hours	72 Hours
Lung	3.63±0.31	3.98±0.05	4.05±0.30	2.82±0.10	2.26±0.46	1.65±0.12	1.44±0.08
Liver	6.48±0.77	6.46±0.13	7.77±1.36	6.34±0.93	2.82±0.45	5.38±1.11	3.40±0.41
Kidneys	16.97±2.73	22.73±0.29	20.15±1.44	10.05±1.41	8.52±1.65	6.26±0.38	8.68±3.60
Ovaries	4.38±0.64	5.13±1.07	5.42±0.16	3.20±0.18	3.66±0.41	2.00±0.17	1.39±0.26
Blood	3.48±0.43	2.40±0.38	3.74±0.32	2.87±0.43	2.01±0.33	1.52±0.23	1.20±0.03
Fat[c]	2.82±0.29	3.38±0.79	1.98±0.13	1.42±0.16	1.77±0.40	0.90±0.06	1.18±0.13

[a] Source: Adapted from Weigel et al., 1978

[b] Values represent mean levels in μg/g of tissue or mℓ of fluid ± S.E.M. of duplicate determinations on three animals at each time interval.

[c] Suprarenal fat

TABLE III-3

Tissue Distribution[a]

Tissue[b]	Oral (2 Rats)		Inhalation (3 Rats, 6-Hour Exposure)			
	100 mg/kg[c] 3 hour Post-Exposure[d] μg Eq/g	$\left(\frac{\text{Tissue}}{\text{Plasma}}\right)$	100 ppm[c] 0 hour Post-Exposure[d] μg Eq/g	$\left(\frac{\text{Tissue}}{\text{Plasma}}\right)$	100 ppm[c] 3 hour Post-Exposure[d] μg Eq/g	$\left(\frac{\text{Tissue}}{\text{Plasma}}\right)$
Stomach	1047.99	(29.01)[e]	6.20±2.19	(0.34)	9.59±2.78	(0.92)
Small intestine	154.53	(4.28)[e]	20.19±10.19	(1.10)	28.99±5.28	(2.79)
Kidney	110.12±7.02	(3.05)	60.40±4.10	(3.30)	42.76±10.79	(4.12)
Large intestine	95.61	(2.65)[e]	75.17±7.50	(4.11)	37.24±22.29	(3.58)
Liver	84.07±13.29	(2.33)	51.34±0.83	(2.81)	42.82±11.95	(4.12)
Pancreas	65.70±1.48	(1.82)	28.44±1.71	(1.55)	20.16±7.21	(1.94)
Lung	64.98	(1.80)[e]	21.30±1.28	(1.16)	13.69±4.50	(1.32)
RBC	42.91±4.15	(1.19)	30.21±2.03	(1.65)	10.69±2.60	(1.03)
Brain	38.17±8.25	(1.06)	19.54±1.27	(1.07)	10.48±4.03	(1.01)
Plasma	36.12±3.38	-	18.29±4.73	-	10.39±2.47	-
Testes	34.69±6.92	(0.96)	16.59±0.66	(0.91)	8.72±2.30	(0.84)
Heart	30.92	(0.86)[e]	17.57±0.88	(0.96)	NS	-
Nasal turbinates	28.91	(0.80)[e]	94.01±18.12	(5.14)	19.79±1.24	(1.90)
Epididymus	24.35±7.21	(0.67)	16.01±1.16	(0.88)	9.47±3.29	(0.91)
Muscle	23.71±6.85	(0.66)	11.67±0.62	(0.64)	5.91±1.61	(0.57)
Fat	10.30±0.18	(0.29)	4.36±2.58	(0.24)	NS	-

[a] Source: Adapted from Smith et al., 1979

[b] Tissues are ranked in decreasing order of tissue/plasma ratios for the oral dose. The concentrations in the tissues are expressed as μg equivalents of epichlorohydrin per gram of tissue and as the ratio of concentration in the tissue to the concentration in the plasma (T/P) at the same time.

[c] Dose level administered or inhalation concentration for 6 hour.

[d] Time rats were killed after dosage or after 6 hour inhalation exposure.

[e] n=1

NS = Not stated

periods of time (Weigel et al., 1978). At 72 hours following dosing, tissue levels ranged from 11% of the 2-hour levels in the adrenals of male rats to 96% in the hearts of females. In general, retention was greater in the tissues of the females than in the males. This data indicates that metabolites of epichlorohydrin may accumulate during chronic dosing. A computer simulation by Smith et al. (1979) indicates that steady-state values would be quickly reached during daily oral dosing, with only minor accumulation of epichlorohydrin and/or metabolites.

Metabolism

Smith et al. (1979) administered [1,3-^{14}C]-epichlorohydrin to male Fisher 344 rats as single oral doses of 1 or 100 mg/kg bw or as 6-hour inhalation exposures to 1 or 100 ppm (~3.78 or 378 mg/m^3). Urinary metabolites were separated by ion-exclusion chromatography. Seven radioactive peaks were found in the urine following oral dosing and six following inhalation exposure, none of which corresponded to epichlorohydrin. The authors noted that the patterns of urinary metabolite excretion were similar following oral or inhalation dosing in the rat except for the detection of one less metabolite following inhalation exposure. Metabolites were not identified.

Jones and Fakhouri (1979) studied the metabolism of several structurally related halopropane derivatives, including epichlorohydrin, in male Sprague-Dawley rats. Urinary metabolites were extracted from acidified urine with ether and identified by co-chromatography with authentic standards on TLC or GLC and by GC-MS. Epichlorohydrin appeared to either react directly with cellular sulfhydryl groups or to be converted to α-chlorohydrin (3-chloro-1,2-propandiol) by an epoxide hydratase. α-Chlorohydrin is in fact, a weak electrophile itself; however, it may be metabolized to the more electrophilic glycidol (1-hydroxy-2,3-epoxypropane), which may react with cellular sulfhydryls.

Jones and O'Brien (1980) reported that the equilibrium for the α-chlorohydrin-to-glycidol reaction strongly favors the formation of α-chlorohydrin; thus, this reaction may occur to only a minor extent in vivo. The reaction rate would probably be limited by the rate at which glycidol is removed by competing reactions, such as conjugation with glutathione. α-Chlorohydrin may also be converted to oxalic acid through a series of oxidations.

Rossi et al. (1983a) found that epichlorohydrin rapidly disappeared from the blood of CD1 mice, with a half-life of ~5 minutes. α-Chlorohydrin appeared in the blood as epichlorohydrin was depleted; however, it had a much longer half-life for disappearance (~50-60 minutes). The relatively long half-life of α-chlorohydrin may contribute to the long biological half-life of ^{14}C, administered as [1,3-^{14}C]-epichlorohydrin, observed in many pharmacokinetic experiments (see Excretion Section). Mohri et al. (1975) have proposed that α-chlorohydrin may also be converted to α-chlorohydrin-1-phosphate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase, within mature sperm. This metabolite is discussed in more detail in Chapter VII.

Andersen et al. (1980) investigated the effect of epichlorohydrin on hepatic nonprotein sulfhydryl (predominantly glutathione) concentrations in male Holtzman rats. They found that a dose of 0.64 mmol/kg bw resulted in a 47% reduction in hepatic glutathione levels, 2 hours post-administration. Thus, glutathione conjugates of epichlorohydrin and/or its metabolites may be major excretory products. This is supported by the work of Jones et al. (1979a,b), who identified two mercapturic acids, S-(2,3-dihydroxypropyl)-cysteine and 1,3-(bis-cysteinyl) propan-2-ol, in the urine of rats (strain unspecified) treated intraperitoneally with 50 mg 1,2-dibromo-3-chloropropane/kg bw in propylene glycol/kg bw, of which epichlorohydrin is a major metabolite.

Epichlorohydrin and glycidol are both electrophilic molecules and can, therefore, react directly with cellular nucleophiles such as glutathione. The reaction of epichlorohydrin with glutathione *in vivo* is, however, catalyzed by glutathione-S-epoxide transferase, thus greatly increasing the rate of the reaction (Fjellstedt et al., 1973; Hayakawa et al., 1975). Fjellstedt et al. (1973) found that this enzyme catalyzed the conjugation of epichlorohydrin with glutathione at 26% of the rate of 1,2-epoxy-3-(p-nitrophenoxy)propane, the standard assay substrate.

Excretion

Weigel et al. (1978) investigated the excretion of radioactivity by male and female CD rats following a single oral dose of 10 mg $[1,3-^{14}C]$-epichlorohydrin/kg bw. The major route of excretion was the urine, accounting for 38-40% of the administered radioactivity over a 72-hour period. In this same time period, expired air accounted for 18-21% of the administered radioactivity and fecal excretion accounted for <4%. The radioactivity in the expired air was predominantly $^{14}CO_2$, with <0.1% of the administered ^{14}C appearing in the expired air as compounds which absorbed to activated charcoal. The excretion of $^{14}CO_2$ was initially very rapid, with the majority of the total $^{14}CO_2$ excretion occurring within 4 hours. Urinary excretion also demonstrated an initial rapid phase of elimination, with the majority of the urinary excretion occurring within 8-12 hours. This data suggests that elimination of [^{14}C]-epichlorohydrin-derived radioactivity may be a biphasic process (Weigel et al., 1978).

Smith et al. (1979) administered single gavage doses of 1 or 100 mg [1,3-^{14}C]-epichlorohydrin/kg bw to groups of four male Fisher 344 rats. In parallel experiments, four rats were exposed (head only) to atmospheres containing 100 ppm (378 mg/m^3) epichlorohydrin for 6 hours. An additional three rats were exposed to atmospheres containing 1 ppm (3.78 mg/m^3) for 6 hours. The rates or routes of excretion were essentially unaffected by either the route of exposure or the dose administered. The urine was the major route of excretion, accounting for 46-54% of the dose. An additional 25-42% was recovered as $^{14}CO_2$ in the expired air. Only 3-6% of the dose was recovered in the feces. Excretion was biphasic, with an initial rapid phase that dominated the first 24 hours post-exposure and a slower second phase that was dominant after 24 hours. The calculated half-lives for elimination from the plasma were 1-2 hours and 26-27 hours for the fast and slow phases, respectively.

Summary

Several studies have indicated that epichlorohydrin is rapidly and extensively absorbed following oral exposure (Weigel et al., 1978; Smith et al., 1979; Rossi et al., 1983a). Information on absorption following exposure by other routes is more limited; however, epichlorohydrin appears to be absorbed following both inhalation (Smith et al., 1979) and dermal (Kremneva and Tolgskaya, 1961) exposures.

Following oral administration of [^{14}C]-epichlorohydrin to rats, peak tissue levels occur after ~2 hours in males and from 2-8 hours in females, depending on the tissue (Weigel et al., 1978). The tissues containing the highest levels of radioactivity are the kidneys, liver, pancreas, spleen and adrenals. Distribution is similar following either oral or inhalation exposures, with the exception of the nasal turbinates and lacrimal glands, which accumulate high levels of ^{14}C following inhalation, but not oral, exposures (Smith et al., 1979). Epichlorohydrin is rapidly removed from blood and is, therefore, not likely to accumulate during chronic exposures (Rossi et al., 1983a). Metabolites of epichlorohydrin, however, are much more persistent and, therefore, have slight potential for accumulation during chronic exposures.

Epichlorohydrin has two electrophilic centers, C1 and C3 and, thus, may bind to cellular nucleophiles such as glutathione. This binding is catalyzed by glutathione-S-epoxide transferase, resulting in a considerable increase in reaction rate (Fjellstedt et al., 1973; Hayakawa et al., 1975). Epichlorohydrin is also the substrate for an epoxide hydratase, resulting in the formation of α-chlorohydrin (Jones and Fakhouri, 1979). α-Chlorohydrin may be oxidized to oxalic acid; converted to glycidol, an electrophilic epoxide, which can then bind to cellular nucleophiles; or be phosphorylated to yield 3-chloroglycerophosphate.

Following either oral or inhalation eposure, epichlorohydrin metabolites are rapidly excreted in the urine and expired air. Urinary excretion is approximately twice as extensive as in excretion in the expired air (Smith et al., 1979; Weigel et al., 1978). Only minor amounts (~4%) are excreted in the feces. Unmetabolized epichlorohydrin has not been detected in any excreta.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as epichlorohydrin from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to epichlorohydrin is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

In the Exposure Estimation Section of this chapter, available information is presented on the range of levels of human exposure and intake for epichlorohydrin from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combinations of intake from those three sources. However, the Summary Section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, for the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. No information was found in either the Federal surveys or state data on measured levels of epichlorohydrin in drinking water from community water supplies. Epichlorohydrin is used as a major raw material for epoxy and phenoxy resins, as well as various polyelectrolytes, and may be used in coagulants or in coatings with the treatment of drinking water. There are no current guidelines for acceptable levels of epichlorohydrin in drinking water.

Diet. No information was obtained on the occurrence of epichlorohydrin in food. Consequently, dietary intake and the contribution of dietary intake to total exposure could not be evaluated.

Air. No information was obtained on ambient air levels of epichlorohydrin. Consequently, respiratory intake and the contribution of respiratory intake to total exposure could not be evaluated.

Summary

No information was available to assess human exposure to epichlorohydrin or to determine the relative source contribution to total exposure by the three common media (drinking water, food and ambient air).

V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Acute Toxicity. The acute toxicity research (studies of 1-14 day duration) of epichlorohydrin is summarized in Table V-1. Epichlorohydrin is both a local irritant and a systemic toxicant when exposure occurs by either the oral, dermal or inhalation routes. Death is due to central nervous system effects or effects on the respiratory center.

Oral Toxicity -- The first reported study of the acute oral toxicity of epichlorohydrin was that of Freuder and Leake (1941). Groups of 15 white mice (strain, sex and age unspecified) were treated with 270 or 588 mg of epichlorohydrin suspended in a 25% aqueous gum arabic solution/kg bw. A dose of 588 mg/kg bw resulted in an initial period of erratic movement, followed by listlessness and reduced respiration. All 15 animals were dead within 24 hours. All mice that received a dose of 270 mg/kg bw survived the 24-hour observation period.

Smyth and Carpenter (1948) determined the acute oral toxicity of epichlorohydrin in Sherman rats (sex unspecified). They reported an LD_{50} of 90 mg/kg bw, but the dose range tested and length of observation were not reported.

Kremneva (1960) and Kremneva and Tolgskaya (1961) administered single oral doses of 250, 325 or 500 mg epichlorohydrin/kg bw in water to groups of 15 rats and 30 mice of unspecified strain and sex. In rats and mice administered 250 mg/kg bw, no deaths occurred within 14 days, and no signs of toxicity were observed. At the two highest doses, death occurred within

TABLE V-1

Acute Toxicity of Epichlorohydrin

Species	Route	Dose Range	Length of Exposure	Effects	Reference
Rat	oral	65, 125 or 250 mg/kg bw	1 dose	Symptoms of renal toxicity (polyuria, proteinuria, reduced urinary chloride) were seen at doses as low as 65 mg/kg bw.	Shumskaya and Karamzina, 1966
Rat	oral	NR	1 dose	LD_{50} = 240 mg/kg bw	Smyth et al., 1962
Rat	oral	NR	1 dose	LD_{50} = 0.22 ml/kg bw (260 mg/kg bw)	Lawrence et al., 1972
Rat	oral	250, 325 or 500 mg/kg bw	1 dose	No signs of toxicity at a dose of 250 mg/kg bw in 2 weeks. Severe toxicity and death at higher doses.	Kremneva, 1960
Rat	oral	NR	1 dose	LD_{50} = 210 mg/kg bw	Weil et al., 1963
Rat	oral	NR	1 dose	LD_{50} = 90 mg/kg bw	Smyth and Carpenter, 1948
Rat	oral	10, 20, 40 and 80 mg/kg bw	5 days/week for 2 weeks	Histologic degeneration observed in the 40 and 80 mg/kg groups. Hematologic changes in all males and in the high-dose females. Increased kidney-to-body weight ratio in all treated gfroups except low-dose males.	Van Esch, 1981
Rat	inhalation	NR	8 hours	4 of 6 died at a concentration of 250 ppm (946 mg/m^3).	Smyth and Carpenter, 1948
Rat	inhalation	120 ppm (454 mg/m^3)	6 hrs/day, 5 days/week, 11 exposures	Lethargy, profuse nasal discharge, weight loss, leucocytosis, congestion, edema, consolidation and inflammation of the lungs, congestion and necrosis of the liver, atrophy and leucocytic reaction in the kidneys; one death.	Gage, 1959
Rat	inhalation	100 ppm (378 mg/m^3)	6 hrs/day, 5 days/week, 9 exposures	Nasal discharge, respiratory irritation, degenerative and necrotic changes in the lungs and kidneys.	Quast et al., 1979b
Rat	inhalation	NR	4 hours	250 ppm (946 mg/m^3) resulted in the death of either 2/6, 3/6 or 4/6.	Carpenter et al., 1949

TABLE V-1 (cont.)

Species	Route	Dose Range	Length of Exposure	Effects	Reference
Rat	inhalation	NR	8 hours	4 of 6 died at a concentration of 250 ppm (946 mg/m^3).	Weil et al., 1963
Rat	inhalation	283-445 ppm (1071-1684 mg/m^3)	6 hours	LC$_{50}$ = 360 ppm (1362 mg/m^3). Mortality occurred within 14 days. Acute respiratory irritation with hemorrhage and edema of the lungs at all dose levels.	Laskin et al., 1980
Rat	inhalation	0.9-9.0 mg/ℓ (900-9000 mg/m^3)	1.5 hours	LC$_{100}$ = 6 mg/ℓ (6000 mg/m^3); LC$_{50}$ ~2.5-4 mg/ℓ (2500-4000 mg/m^3); NOEL = 1.2 mg/ℓ (1200 mg/m^3).	Kremneva, 1960
Rat	inhalation	NR	4 hours	LC$_{50}$ = 631 ppm (2388 mg/m^3)	Grigorowa et al., 1977
Rat	i.p.	NR	1 dose	LD$_{50}$ = 0.10-0.14 mℓ/kg bw (118-165 mg/kg bw)	Lawrence et al., 1972
Rat	s.c.	65-250 mg/kg bw	1 dose	Symptoms of renal toxicity (polyuria, proteinuria) were observed at all doses.	Shumskaya and Karamzina, 1966
Rat	s.c.	100-180 mg/kg/bw	1 dose	LD$_{50}$ = 125 mg/kg bw. Oliguria was observed at all doses. Changes in lung and kidney histology at dose of 180 mg/kg bw.	Pallade et al., 1967
Rat	s.c.	150 and 180 mg/kg/bw	1 dose	Reduced blood catalase. Lung and kidney pathology, oliguria, anuria.	Rotaru and Pallade, 1966; Pallade et al., 1967
Rat	dermal	0.5-2.0 mℓ/kg bw (590-2360 mg/kg bw)	1-4 doses (1 dose/day)	A single application of 590 mg/kg bw was not lethal. 4 consecutive daily doses of 590 mg/kg bw were 100% lethal.	Freuder and Leake, 1941
Mouse	oral	NR	1 dose	LD$_{50}$ = 0.20 mℓ/kg bw (236 mg/kg bw).	Lawrence et al., 1972
Mouse	oral	250-500 mg/kg bw	1 dose	No deaths at 250 mg/kg bw. LD$_{100}$ = 325 mg/kg bw.	Kremneva, 1960
Mouse	oral	0.08-0.50 mℓ/kg bw (94-590 mg/kg bw)	1-21 daily doses	Single dose LD$_{100}$ = 590 mg/kg bw. Daily doses of 94 mg/kg bw killed all mice within 21 days.	Freuder and Leake, 1941

TABLE V-1 (cont.)

Species	Route	Dose Range	Length of Exposure	Effects	Reference
Mouse	inhalation	237-2124 ppm (897-8038 mg/m³)	2 hours	No deaths at 316 ppm (1196 mg/m³). 40% mortality at 590 ppm (2233 mg/m³).	Kremneva, 1960
Mouse	inhalation	NR	2 hours	LC_{50} = 789 ppm (2986 mg/m³)	Grigorowa et al., 1977
Mouse	inhalation	2370, 8300 and 16,600 ppm (8969, 31,410 and 62,822 mg/m³)	0.5-1.0 hour	Irritation of nose and eyes at all concentrations. 100% mortality at exposures of ≥31,411 mg/m³ for 0.5 hours.	Freuder and Leake, 1941
Mouse	inhalation	71,890 mg/m³	NR	LT_{50} = 9.13 minutes	Lawrence et al., 1972
Mouse	inhalation	100 ppm (378 mg/m³)	6 hrs/day, 5 days/week, 9 exposures in 12 days	Nasal irritation, slight thymic atrophy, decreased food consumption and body weight, inflammation and degeneration changes in nasal turbinates, decreased hepatic glycogen, decreased hepatocyte size, focal subacute pneumonites.	Quast et al., 1979b
Mouse	i.p.	NR	1 dose	LD_{50} = 170 mg/kg bw	Lawrence et al., 1972
Mouse	dermal	pure	15-20 minutes	70% mortality in 24 hours.	Pallade et al., 1967
Mouse	dermal	pure	20-30 minutes for 2-3 exposures	100% mortality	Kremneva and Tolgskaya, 1961
Guinea pig	i.p.	NR	1 dose	LD_{50} = 0.10-0.14 mℓ/kg bw (118-165 mg/kg bw).	Lawrence et al., 1972
Guinea pig	dermal	4800 mg/kg bw	1 hour	No deaths were observed.	Freuder and Leake, 1941
Guinea pig	mixed	5% in ethanol	intradermal injection, 1 week rest, dermal (48 hours)	Contact sensitivity developed in 9 of 15 (60%) of the animals.	Thorgeirsson and Fregert, 1977

TABLE V-1 (cont.)

Species	Route	Dose Range	Length of Exposure	Effects	Reference
Rabbit	i.p.	NR	1 dose	LD_{50} = 0.10-0.14 mℓ/kg bw (118-165 mg/kg bw)	Lawrence et al., 1972
Rabbit	dermal	NR	24 hours	LD_{50} = 1540 mg/kg bw	Smyth et al., 1962
Rabbit	dermal	NR	1 dose	LD_{50} = 1.3 mℓ/kg bw (1534 mg/kg bw)	Weil et al., 1963
Rabbit	dermal	118-590 mg	2-24 hours	Exposure to 590 mg for 24 hours produced edema and necrosis.	Pallade et al., 1967
Rabbit	dermal	NR	1 dose	LD_{50} = 0.64 mℓ/kg bw. Local irritation at concentrations of 0.625% in cottonseed oil or higher.	Lawrence et al., 1972
Rabbit	dermal	NR	1 dose	LD_{50} = 1.3 mℓ/kg bw (1534 mg/kg bw).	Smyth and Carpenter, 1948
Rabbit	corneal	1 drop	1 dose	Corneal clouding and swelling.	Kremneva and Tolgskaya, 1961
Rabbit	corneal	1 drop	1 dose	Grade 4 eye irritant, grades not clearly defined, but range from 1 to 10.	Weil et al., 1963
Rabbit	corneal	5-80% in cottonseed oil	0.1 mℓ/eye	The minimum concentration which resulted in irritation was 10%.	Lawrence et al., 1972
Cat	i.v.	9.3-93 mg/kg bw	1 dose	Blood pressure decrease at a dose of 9.3 mg/kg bw.	Freuder and Leake, 1941
Dog	i.v.	9.3-93 mg/kg bw	1 dose	Blood pressure decrease at a dose of 9.3 mg/kg bw.	Freuder and Leake, 1941

NR = Not reported

i.p. = intraperitoneal

i.v. = intravenous

s.c. = subcutaneous

48 hours. Gross symptoms included decreased mobility, labored breathing, rales in the lungs, marked hyperemia of the skin and subcutaneous hemorrhage, ataxia, periodic tremors of the body and a distention of the stomach. At necropsy, all the internal organs and cerebrum were hyperemic, hemorrhaging was observed in the lungs, the liver was yellow and distended and the stomach and intestines were filled with gas. Histologic examination revealed dystrophic changes in the epithelium of the convoluted tubules of the kidneys, with sites of necrosis in the individual tubules. The liver had vacuolization of the hepatic cells with small droplets of fatty dystrophy. Necrotic foci were observed in the gastric and intestinal mucosa.

Smyth and coworkers (Smyth et al., 1962; Weil et al., 1963) reported an acute oral LD_{50} for undiluted epichlorohydrin in male Carworth-Wistar rats (90-120 g bw). Due to ambiguities in the data presentation, it is not clear whether the LD_{50} is 0.21 kg/kg bw (range 0.20-0.23 kg/kg bw) or 0.21 mℓ/kg bw (~0.26 g/kg bw). No data on the dose ranges tested or the length of observation were presented.

Lawrence et al. (1972) reported acute oral LD_{50}s for epichlorohydrin given in cottonseed oil to male Sprague-Dawley rats and male ICR mice of 0.22 and 0.20 mℓ/kg bw, respectively. The 95% confidence intervals for the LD_{50}s were 0.1615-0.2476 mℓ/kg bw for mice and 0.1242-0.3908 mℓ/kg bw for rats. Animals were observed for 7 days after dosing; however, no data on the time to death were presented.

Van Esch (1981) treated five male and five female Wistar rats by gavage with epichlorohydrin in water at doses of 0, 1, 2, 4 and 8 mg/100 g bw, 5 days/week for 2 weeks. The only deaths were one female in each of the two highest dosed groups. In addition, females in the highest dosed group had poor body weight gain (26 vs. 11 g), and hematocrit, hemoglobin and erythrocyte counts were decreased, while mean corpuscular volume and hemoglobin were increased significantly ($p<0.05$). In males, body weight gain was not affected, while decreased erythrocyte counts were reported for all dose groups and decreased hemoglobin in the ≥20 mg/kg group, and decreased hematocrit in the ≥40 mg/kg group were reported. This is shown in Table V-2. In both sexes, degenerative changes were observed upon histological examination of the kidneys in the 40 and 80 mg/kg groups.

Absolute organ weights were unaffected by treatment of the rats with epichlorohydrin. However, organ/body weight ratios were significantly increased ($p<0.05$ or better) in treated groups as follows: liver and heart in 80 mg/kg males and females; brain and thymus in 80 mg/kg females; brain in 10 mg/kg females; spleen in 70 mg/kg males; kidney in all treated groups of females and the three highest treated groups of males. Evidence of a dose relationship of the effect of epichlorohydrin on kidney is shown in Table V-3.

Dermal Toxicity -- Freuder and Leake (1941) applied pure epichlorohydrin to the clipped abdomens of rats and guinea pigs (strains and sex unspecified). Doses of 0.5, 1.0 or 2.0 mℓ/kg bw (590, 1180 or 2360 mg/kg bw) for rats, and 4800 mg/kg bw for guinea pigs were applied to a 1 cm^2 piece of gauze and held in place by a double layer of adhesive plaster. The gauze was removed after 1 hour. Discoloration and superficial desquamation were noted within a few hours of exposure. Repeated daily exposure to 0.5

TABLE V-2

Results of Hematological Analysis for Male Rats Given Epichlorohydrin Orally for 10 Days[a]

		mg Epichlorohydrin/kg bw			
	Control	10	20	40	80
Number of Animals	5	5	4	5	5
Hemoglobin mmol/ℓ	9.7	9.4	9.2[b]	9.3[b]	8.7[c]
Hematocrit ℓ/ℓ	0.454	0.439	0.445	0.442[b]	0.415[c]
Erythrocytes 10^{12}/ℓ	7.36	6.99[b]	6.92[d]	7.13	6.77[c]

[a] Source: Adapted from Van Esch (1981)

[b] $p<0.05$

[c] $p<0.001$

[d] $p<0.01$

TABLE V-3

Average Absolute and Relative (as % of body weight) Kidney Weights in Male and Female Wistar Rats Given Epichlorohydrin by Gavage for 10 Days[a]

	mg Epichlorohdyrin/kg bw				
	0	10	20	40	80
Number of Animals	5	5	4 or 5	4 or 5	4 or 5
Females					
Body Weight (g)	149	147	146	145	130
Kidney Weight (mg)	1150	1268	1275	1478	2467
Kidney:Body Weight Ratio	0.769[b]	0.862[b]	0.872[b]	1.015[b]	1.862[c]
Males					
Body Weight (g)	198	206	215	196	193
Kidney Weight (mg)	1443	1619	1759	1749	2110
Kidney:Body Weight Ratio	0.730	0.785	0.833[b]	0.893[b]	1095[b]

[a] Adapted from Van Esch, 1981

[b] $p<0.001$

[c] $p<0.01$

or 1.0 mℓ/kg bw for 2 to 4 applications produced superficial necrosis exceeding the size of the gauze, with the skin becoming dry, parchment-like and brown. These effects gradually disappeared when the animals survived. Systemic effects included decreased movement, bristled hair and depressed respiration. Even the lowest dose (590 mg/kg bw) produced 100% mortality in rats after four doses. All guinea pigs survived both single and repeated exposures.

Smyth and Carpenter (1948) determined the acute dermal toxicity of epichlorohydrin to rabbits (strain and sex unspecified). They reported an LD_{50} of 1.3 mℓ/kg bw (~1.54 g/kg bw), but did not give any indication of the range of doses tested or the length of observation. This value has also been reported by Smyth et al. (1962) and Weil et al. (1963).

Kremneva and Tolgskaya (1961) immersed the tails of groups of 10 mice (strain and sex unspecified) in epichlorohydrin for 1 hour or 2-3 times for 20-30 minutes per time. In the animals exposed once for 1 hour, lethargy, depressed respiration and loss of righting reflex were observed. Congestion and edema occurred in many (unspecified) internal organs. Other pathology included renal tubular necrosis and hemorrhages in the brain. Six of the 10 mice in this group died, but the time to death was not reported. The repeated exposures resulted in the deaths of all 10 mice. In a similar experiment, Pallade et al. (1967) immersed the tails of 10 mice (strain and sex unspecified) in epichlorohydrin for 15-20 minutes. This treatment resulted in the death of 7 of the 10 mice within 24 hours.

Kremneva and Tolgskaya (1961) also investigated the effect of epichlorohydrin exposure on rabbit skin. A small glass cap, containing 0.5-1.0 mg (0.42-0.85 μℓ) epichlorohydrin, was attached to the back of a rabbit (strain and sex unspecified) for 1 hour. The skin became hyperemic, followed by the development of ulcer-like lesions and scabbing. Gradual recovery occurred over a period of 1-1.5 months.

Lawrence et al. (1972) measured the acute dermal LD_{50} and irritant activity of epichlorohydrin in New Zealand rabbits (sex unspecified). The material to be tested, either epichlorohydrin or epichlorohydrin diluted with cottonseed oil, was applied to a small patch and placed on the shaved back of the rabbit. The patch was then covered with an occlusive bandage for 24 hours, at which time the bandage and patch were removed and observation continued for up to 6 days. The acute dermal LD_{50} in this study was 0.64 mℓ/kg bw (~0.755 g/kg bw) with a 95% confidence interval of 0.33-1.22 mℓ/kg bw. Death occurred within 6 days. When irritant activity was assessed on a scale of 0-3+, undiluted epichlorohydrin was at least as irritating as the positive control, an 8% aqueous solution of sodium lauryl sulfate (3+). The lowest concentration of epichlorohydrin which evidenced any irritating activity was 0.625% (v/v) in cottonseed oil.

Several groups (Lawrence et al., 1972, 1974; Weil et al., 1963; Thorgeirsson and Fregert, 1977) have investigated the sensitization potential of epichlorohydrin using the guinea pig maximization test. In this test, sensitization is induced by intradermal injections of the test compound, sometimes accompanied by dermal applications. Two to 3 weeks following the last sensitization treatment, the test chemical is applied to a shaved path on the skin and the animals are observed for sensitization reactions over the next 24-48 hours. No evidence of sensitization was found by Lawrence et al. (1972, 1974) or Weil et al. (1963); however, Thorgeirsson and Fregert (1977) obtained positive results in 9 of 15 animals tested. This may indicate a sex difference, since Thorgeirsson and Fregert used female Hartley guinea pigs while Lawrence et al. used males. Weil et al. (1963) did not specify the strain or sex of their animals. Overall, the data are too sketchy to support any conclusion as to the sensitization potential of epichlorohydrin.

Inhalation Toxicity -- Freuder and Leake (1941) exposed groups of 20-30 white mice (strain and sex unspecified) to atmospheres containing 2370, 8300 or 16,600 ppm epichlorohydrin (8969, 31,411 or 62,822 mg/m^3) for 30 minutes at the low dose and 60 minutes at the two higher doses. Irritation of the nose and eyes was produced immediately at all doses. At higher concentrations, "delirium" was seen within 3-14 minutes. Occasional animals showed excitement just before removal at the end of the 30-minute exposure period. Toxic symptoms included a gradually developing cyanosis with subsequent muscular relaxation of the extremities. Later the tail stiffened and fine tremors of the body developed. Respiratory depression developed shortly before death, which occurred several days following exposure. No mice survived exposure to 31,411 or 62,822 mg/m^3 for 30 minutes. A single 60-minute exposure to 8969 mg/m^3 did not kill any of the exposed mice; however, 16 consecutive daily exposures to this concentration produced 100% mortality.

In a range finding study, Smyth and Carpenter (1948) reported that four of six Sherman rats died following an 8-hour exposure to atmospheres containing 250 ppm (946 mg/m^3) epichlorohydrin. The time to death was not reported. In another range-finding study, Carpenter et al. (1949) found that exposure to atmospheres containing 250 ppm (946 mg/m^3) for 4 hours resulted in the death of two to four Sherman rats/group of six exposed. Concentrations in these studies were calculated, and not determined analytically.

Gage (1959) exposed groups of four male and four female Wistar rats to atmospheres containing 120 ppm (454 mg/m^3), 6 hours per day, 5 days per week, for a total of 11 exposures. Labored breathing developed within the first 3 hours of exposure and continued throughout the study. The rats became progressively lethargic, and their condition deteriorated noticeably as treatment continued. A profuse nasal discharge developed by the end of the fourth treatment. Marked leucocytosis, urinary protein excretion and weight loss developed during the course of exposure, with one rat dying after the 11th eposure. At necropsy, gross and histopathological lesions were observed in the lungs, livers and kidneys. Gross observation revealed patchy discoloration of the lungs, blotchy livers and pale kidneys. Histological examination of the lungs revealed congestion, edema, consolidation, inflammation and abscess formation. The livers were congested, with one liver displaying areas of necrosis. Leucocytic reactions were found in all kidneys, with atrophy of the peripheral cortical tubules occurring in four rats. In parallel experiments with New Zealand rabbits, nasal irritation was observed after two exposures to 16 ppm (61 mg/m^3). These rabbits were continued on treatment for 20 days, but the concentration was decreased to 9 ppm (34 mg/m^3) after the second day. No tissue damage was detectable by the end of the treatment period.

Kremneva (1960) and Kremneva and Tolgskaya (1961) exposed 70 mice and 62 rats (strains and sex unspecified) to various atmospheres containing 0.9-1.2, 2.5-4.0, 4.5-6.0, 6.0-7.0 and 7.5-9.0 mg epichlorohydrin/ℓ (900-9000 mg/m^3) for 2 hours. The maximum concentration that did not produce any gross signs of toxicity was 1200 mg/m^3. Exposure to 2500-4000 mg/m^3 produced 40% mortality in mice within 14 days, and at exposures of 6000 mg/m^3 or higher, mortality was 100% within 1 day. Rats displayed approximately the same sensitivity to epichlorohydrin as did mice. Initial toxic symptoms included irritation of the mucous membranes of the upper respiratory tract and motor stimulation. Later, the animals became immobile, developed dyspnea alternating with slow difficult breathing and suffered periodic tremors. Death occurred several hours to days after exposure. At necropsy, the stomach and intestines were distended and filled with gas, with occasional hemorrhaging of the mucous membranes of the stomach and small intestine. Pulmonary edema, small hemorrhagic foci and bronchitis were observed in the lungs. Histological examination of the lungs revealed catarrhal desquamative bronchitis, peribronchitis, necrotic foci of the bronchial mucosa and pulmonary edema. Protein dystrophy of the epithelium of the renal convoluted tubules, with necrotic foci and protein adhesions in the tubular lumen, was observed in the kidneys. Edema was found in the capsules of the individual glomeruli. Even though no signs of toxicity were observed following a single 2-hour exposure to 1000 mg/m^3, five exposures over a 2-week period were lethal to 1 of 5 rats and 2 of 10 mice.

Lawrence et al. (1972) bubbled air through epichlorohydrin and into a 8.75 ℓ glass exposure chamber. Based on the weight loss of the liquid and the volume of air passed through, the calculated epichlorohydrin concentration was 71.89 mg/ℓ (71,890 mg/m³). Under these conditions, the LT_{50} was 9.13 minutes, with 95% confidence limits of 8.49-9.81 minutes. At this time, the air in the chamber would have reached ~88% of equilibrium.

Quast et al. (1979b) exposed groups of five male and five female Fisher 344 rats, Sprague-Dawley rats, or $B6C3F_1$ mice to atmospheres containing 100 ppm (380 mg/m³) epichlorohydrin for 6 hours/day, 5 days/week, for a total of nine exposures in 12 days. The concurrent control group consisted of five male and five female rats. The animals demonstrated a marked tendency to huddle together and sleep during the exposure periods. No signs of eye or nasal irritation were noted during the exposures, but the rats were observed to have a slight nasal discharge upon removal from the exposure chamber. Signs of respiratory distress, an apparent decrease in food consumption and reduced fecal excretion were noted during the exposure periods, with partial recovery over the weekend. Gross effects of this treatment included decreased body weight gain, changes in the nasal mucosa, leukocytosis secondary to nasal inflammation, decreased urinary specific gravity and, in rats only, increased kidney weight. Hematologic and urinary examinations were conducted on rats only.

Histopathological changes in the nasal turbinates were characterized by degeneration, inflammation, hyperplasia and squamous metaplasia. These changes occurred in both species, but were less severe in mice than in rats. Minimal treatment-related changes (decrease hepatocellular glycogen content, decreased hepatocyte size and increased variability in cytoplasmic staining) were observed in both species. Degenerative changes were noted in the kidneys of both strains of rats, but not in the mice, indicating that rats were more sensitive than mice in this study to the toxic effects of epichlorohydrin (Quast et al., 1976b).

Laskin et al. (1980) exposed six groups of 20 male Sprague-Dawley rats for 6 hours to atmospheres containing 283-445 ppm (1171-1648 mg/m³) epichlorohydrin and recorded the mortality over a 14-day period. This study yielded an LC_{50} of 360 ppm (1362 mg/m³). Four animals from each group were autopsied, revealing acute respiratory irritation with hemorrhage and severe edema of the lungs, resulting in an increased lung-to-body weight ratio. The results of this study are summarized in Table V-4.

Other Routes of Exposure --

Corneal. Several groups have measured the local effects of epichlorohydrin on the rabbit eye (Kremneva and Tolgskaya, 1961; Weil et al., 1963; Lawrence et al., 1972). Weil et al. (1963) classify epichlorohydrin as a grade 4 eye irritant; however, their grading system is not clearly defined. Grade 1 is defined as "at most very small area of necrosis resulting from 0.5 mℓ of the undiluted chemical"; grade 5 "indicates a so-called severe burn from 0.005 mℓ" (Smyth et al., 1962).

Lawrence et al. (1972) applied 0.1 mℓ epichlorohydrin in cottonseed oil to the superior temporal quadrant of the right eye of New Zealand rabbits and examined the eye every 30 minutes for 3 hours. The results of

TABLE V-4

Acute Inhalation Toxicity of Epichlorohydrin[a]

Exposure Level (ppm)[b]	Number of Deaths Within 14 Days of Exposure	Percent Increase Lung/Body Weight Ratio
445	17	66
421	16	36
369	15	80
339	1	14
303	1	0
283	0	0

[a] Source: Adapted from Laskin et al., 1980

[b] 20 rats per exposure level

this test are presented in Table V-5. Under these conditions, a 20% solution of epichlorohydrin in cottonseed oil represented the lowest effect level for eye irritation, whereas the effect with a 10% solution was questionable.

Cytotoxicity. Lawrence et al. (1972) investigated the cytotoxicity of epichlorohydrin to mouse fibroblast cells (NCTC clone 929, Strain L, Earle) in culture. Using the agar-overlay technique, they found a concentration of 0.001215% (v/v) epichlorohydrin was cytotoxic to the L-cells, but 0.000486% (v/v) was not cytotoxic when incubated for 24 hours. When evaluated for its effect to inhibit growth of L-cells in culture for 72 hours, a dose-response curve indicated that a concentration of 1.6×10^{-5} moles/ℓ in the medium reduced growth of the L-cells by 50%.

Intravenous Toxicity. Freuder and Leake (1941) investigated the intravenous toxicity of epichlorohydrin in three dogs and two cats. The epichlorohydrin was injected in either water or acacia solution/suspension, but the procedure by which these animals were treated to obtain the reported dose response was not described. A dose of 93 mg/kg bw resulted in a rapid decrease in blood pressure, followed by a slight increase in both species. In dogs, but not cats, there was a brief period of apnea immediately following dosing. In both species, the respiration rate increased markedly, and death occurred within 2 hours. A dose of 9.3 mg/kg bw resulted in only transient decreases in blood pressure. At doses below 9.3 mg/kg bw, no effect of intravenous epichlorohydrin was noted.

TABLE V-5

Corneal Irritation Due to Epichlorohydrin[a]

Concentration[b]	Response
5%	no irritation
10%	doubtful irritation
20%	definite conjunction and palpebral irritation with significant edema
40%	iritis and palpebral irritation with significant edema
80%	corneal damage

[a] Source: Adapted from Lawrence et al., 1972

[b] Solvent was cottonseed oil.

Intraperitoneal Toxicity. Lawrence et al. (1972) has reported the acute intraperitoneal toxicity of epichlorohydrin in cottonseed oil in male ICR mice, male Sprague-Dawley rats, male Hartley guinea pigs and male New Zealand rabbits. Epichlorohydrin was more toxic to rats (LD_{50}=113 mg/kg bw) than to mice (LD_{50}=170 mg/kg bw). Toxicity in rabbits (LD_{50}=160 mg/kg bw) and guinea pigs (LD_{50}=118 mg/kg bw) was highly variable, with 95% confidence limits for the LD_{50}s of 29.5-472 mg/kg bw for guinea pigs and 83.6-306 mg/kg bw or rabbits.

Subcutaneous Toxicity. The subcutaneous toxicity of epichlorohydrin was first reported by Kremneva and Tolgskaya (1961), who administered 0, 125, 250, 375 or 500 mg epichlorohydrin/kg bw to groups of 10 mice (strain and sex unspecified). Doses of 500 or 375 mg/kg bw resulted in the deaths of all mice. A dose of 250 mg/kg bw resulted in the death of 7/10 mice, and no physical or behavioral changes were observed at a dose of 125 mg/kg bw.

Shumskaya and Karamzina (1966) investigated the effects of subcutaneously administered epichlorohydrin on the kidneys of rats (strains and sex unspecified). They reported an increase in nitrogenous substances in the blood, proteinuria, decreased urinary chlorides, decreased urinary specific gravity, polyuria and increased kidney-to-body weight ratios following doses of 65, 125 or 250 mg epichlorohydrin/kg bw, but no dose-response information was reported.

The acute effects of subcutaneously administered epichlorohydrin were more thoroughly investigated by Rotaru and Pallade (1966) and Pallade et al. (1967). They administered a single dose of 150 mg epichlorohydrin to 23 rats (180-200 g) and 180 mg to 14 rats (180-200 g) of unspecified strain and sex. Animals were sacrificed after 1, 2, 5 or 10 days, and the heart, lungs, kidneys, liver, adrenals, spleen, stomach, intestine and brain were removed for histological examination. At both doses, toxic effects were observed in the kidneys, consisting of ischemia of the cortex, congestion of the medulla with marked interstitial edema, degenerative changes throughout the tubules and necrotic lesions of the proximal convoluted tubules. These effects were more pronounced with the 180 mg/kg dose. Regeneration was observed by 5 days and by 10 days most of the tubular integrity was restored and only slight ischemic necrosis was observed. Congestion was observed in all tissues examined. Desquamative bronchial inflammation and edema of the bronchiovascular connective tissue were observed in the lungs. Stasis and hemorrhage occurred in the spleen, and edema was observed in the stomach and intestines.

Chronic and Subchronic Toxicity. The chronic and subchronic toxicities of epichlorohydrin are summarized in Table V-6. The pathological changes observed are qualitatively similar to those observed following acute exposures.

Oral Toxicity -- In the study of Freuder and Leake (1941), groups of 15 mice (strain, sex and weight unspecified) were administered daily oral doses of 94 mg, 190 mg or 271 mg epichlorohydrin in 25% aqueous gum arabic/kg bw until all animals were dead. All animals in these dose groups

TABLE V-6

Chronic and Subchronic Toxicity of Epichlorohydrin

Species	Route	Dose Range	Length of Exposure	Effects	Reference
Rat	oral	2 and 10 mg/kg bw	5 days/week for 104 weeks	A dose-related decrease in white blood cells was observed, and an increase in hyperplasia of the stomach at both exposure levels	Van Esch, 1981, 1982
Rat	inhalation	170-250 mg/m^3 (This exposure was reported as a range.)	3 hours/day, for 4 months	Two deaths after 1 month. 100% Mortality after 4 months. Elevated blood pressure after 1-2 months. General deterioration in condition with time. Respiratory distress and dyspnea, bronchitis. Necrosis of bronchial mucosa. Deterioration of kidneys with necrosis of convoluted tubules. Fatty degeneration and vacuolization of hepatic cells. Irregular staining of myocardial tissue, fragmentation of myocardial fibers.	Kremneva and Tolyskaya, 1961
Rat	inhalation	0.2, 2 and 20 mg/m^3	continuous for 98 days	No effects observed at 0.2 mg/m^3. 2 mg/m^3 resulted in decreased blood nucleic acids, but no abnormal pathology. 20 mg/m^3 produced emphysema, desquamative interstitial pneumonia, edema and deterioration of vascular connective tissues of the lung and extensive damage to heart and kidney tissue.	Fomin, 1966, 1968
Rat	inhalation	5, 25 and 50 ppm (19-189 mg/m^3)	6 hours/day, 5 days/week, 61-62 exposures	No effects observed at 19 mg/m^3. Dose-related pathology, including decreased activity, eye irritation and degenerative changes in respiratory and olfactory epithelium were observed at 95 and 189mg/m^3. Extensive pathological changes in the kidney occurred at 189 mg/m^3.	Quast et al., 1979a
Rat	inhalation	100 ppm (378 mg/m^3)	6 hours/day for 30 days	Inflammation of the nasal turbinates, edema, congestion and pneumonia in the lungs, dilatation of cortical and medullary tubules in the kidney.	Laskin et al., 1980

TABLE V-6 (cont.)

Species	Route	Dose Range	Length of Exposure	Effects	Reference
Rat	inhalation	10 and 30 ppm (38 and 114 mg/m^3)	6 hours/day 5 days/week for the rats lifetime	Pulmonary congestion, bronchiolectasis, pneumonia, tubular degenerative changes with dilatation, flattening of the tubular epithelium and some atrophy were observed in the kidneys.	Laskin et al., 1980
Rat	inhalation	5, 25 and 50 ppm (19-189 mg/m^3)	6 hours/day, 5 days/ week, 10 weeks	Infertility in males at 189 mg/m^3, decreased fertility at 95 mg/m^3. Irritation of nasal turbinates. No effects at 19 mg/m^3.	John et al., 1983
Rat	i.p.	0.00955-0.01910 mℓ/kg bw	daily for 30 days	Pulmonary lesions, decreased weight gain, increased kidney/bw ratio.	Lawrence et al., 1972, 1974
		0.04774 mℓ/kg bw	3 days/week for 12 weeks	Increased heart, kidney and liver/bw ratios, decreased food consumption and altered blood chemistry.	
		0.0095-0.0190 mℓ/kg bw	3 days/week for 12 weeks	Altered blood chemistry, decreased food consumption at the high dose.	
Mouse	oral	94, 190 and 271 mg/kg bw/day	21 days	271 mg/kg bw resulted in 100% mortality after 4 doses. 94 mg/kg bw resulted in 100% mortality after 21 doses.	Frueder and Leake, 1941
Mouse	inhalation	2370 ppm (8968 mg/m^3)	1 hour/day until death	50% mortality after 7 exposures, 100% mortality after 16 exposures.	Freuder and Leake, 1941
Mouse	inhalation	5, 25 and 50 ppm (19-189 mg/m^3)	6 hours/day, 5 days/ week, 61-62 exposures	No effects at 19 mg/m^3. Decreased weight gain, inflammation and degenerative changes in nasal turbinates at 95 mg/m^3. Focal subacute pneumonitis appeared at 189 mg/m^3.	Quast et al., 1979a
Rabbit	inhalation	9 and 35 ppm (34 and 132 mg/m^3)	6 hours/day for 20 days	Nasal irritation but no pathological changes.	Gage, 1959
Rabbit	inhalation	5, 25 and 50 ppm (19-189 mg/m^3)	6 hours/day, 5 days/ week for 10 weeks	Irritation of the nasal turbinates at 25 and 50 ppm. No effects on fertility.	John et al., 1983
Dog	s.c.	8 mg/kg bw/day	30 days	Dosed with α-chlorohydrin, the major metabolite of epichlorohydrin. Inhibition of RNA and sialic acid synthesis in epididymides.	Dixit, 1977

died by days 21, 8 and 4, respectively. At the lowest dose, the first death occurred on day 2. At the higher doses, the first death occurred within 24 hours of the initial treatment. Death was preceded by decreased activity, muscular relaxation of the extremities, stiffening of the tail, fine tremors, depressed respiration and clonic convulsions.

In a report describing preliminary results, Van Esch (1981, 1982) administered 0, 2 and 10 mg epichlorohydrin/kg bw in water by gavage to weanling Wistar rats 5 days/week for 104 weeks. Each group consisted of 50 males and 50 females. No treatment-related changes were observed in growth in either sex until the last 4-6 weeks when males lost considerable weight. Early mortality in all groups was attributed to intestinal blockage by "hairballs"; however, the mortality was significantly higher in the treated animals. A change to a diet richer in fiber at 8 months eliminated further deaths resulting from hairballs. The only treatment-related effect on hematology was a dose-related decrease in white blood cells in females (8060, 7430 and 6560×10^{6} cells/ℓ in the control, low- and high-dose groups, respectively). These changes were not statistically significant. Histologic examination has only been completed on the stomachs, in which hyperplastic lesions were observed in 3/50, 12/50 and 7/49 females, and 5/50, 24/50 and 6/50 males in the control, low- and high-dose groups. The apparent decrease in hyperplasia at the high-dose levels occurs because all of the animals dying or sacrificed after 26 months of exposure had carcinoma of the forestomach and were not classified as hyperplastic but were placed in the carcinoma category.

Inhalation Toxicity -- In contrast to chronic oral toxicity, the chronic inhalation toxicity of epichlorohydrin has been extensively studied. Freuder and Leake (1941) exposed groups of 10 white mice (strain, sex and weight unspecified) to atmospheres containing 0.1 mM epichlorohydrin/ℓ (9250 mg/m³) for 1 hour per day until all animals were dead (16 days). Irritation of the nose and eyes occurred within two treatments. The mice then evidenced decreased activity and muscular relaxation of the extremities. Respiration was depressed and became increasingly labored with continuing exposure. Clonic convulsions were commonly noted preceding death.

Gage (1959) exposed groups of four male and four female Wistar rats (160-200 g) to atmospheres containing 9-56 ppm epichlorohydrin (34, 64, 102 and 212 mg/m³) 6 hours per day, 5 days per week, for at total of 18-19 exposures. A fifth group was exposed to 120 ppm (456 mg/m³) for a total of 11 exposures. Eighteen exposures to 34 mg/m³ produced no detectable effects. After 19 exposures to 64 mg/m³, the animals appeared to be in slightly inferior condition; however, no lesions were observed on gross or histological examination. Eighteen exposures to 102 mg/m³ produced mild nasal irritation, but had no effect on body weight gain. Histological examination revealed areas of hemorrhage and consolidation in the lung of one rat (sex unspecified). In the 212 mg/m³ group, lethargy and respiratory distress were observed after 10 exposures. A nasal discharge developed during the later exposures. The rats lost weight during the week, but demonstrated partial recovery over the weekends. In the 456 mg/m³ group, effects similar to those in the 212 mg/m³ group were observed except the severity was increased, and the animals did not recover on weekends. Histological examination revealed congestion in the lungs, livers and kidneys.

No gross or histological lesions attibutable to epichlorohydrin exposure were found at necropsy in any group except the 456 mg/m³ group. No control group was indicated.

Gage (1959) also exposed groups of two male New Zealand rabbits to atmospheres containing 9-35 ppm (34-132 mg/m³) epichlorohydrin, 6 hours per day, 5 days per week, for a total of 20 days. Twenty exposures to 132 mg/m³ produced signs of nasal irritation, but no loss of body weight. Two rabbits given two exposures of 61 mg/m³ also produced signs of nasal irritation. The exposure concentration was then lowered to 34 mg/m³ and continued for 20 exposures. No signs of nasal irritation were noted at the lower concentration. No gross or histological lesions were found at necropsy following either level of exposure.

Kremneva and Tolgskaya (1961) exposed groups of eight rats (strains, sex and weight unspecified) to atmospheres ranging from 0.17-0.25 mg epichlorohydrin/ℓ (170-250 mg/m³) 3 hours per day for 4 months. After 1-2 months of exposure, several animals developed subcutaneous hemorrhage, hyperemia of the skin, elevated blood pressure, respiratory difficulties and dyspnea. Two rats died during the second month. During the third month, the condition of the animals appeared to improve slightly; however, they deteriorated rapidly during the fourth month, and all rats were dead by the start of the fifth month. Histological examinations at the time of death revealed lesions of the lungs, kidneys, liver and heart. The lesions in the respiratory tract included bronchitis, necrosis of the bronchial mucosa, thickened alveolar septa and pulmonary edema. The deterioration of the kidneys was characterized by necrosis of the convoluted tubules. Damage in the liver consisted of fatty degeneration and vacuolization. Myocardial tissue demonstrated irregular staining with fragmentation of the myocardial fibers.

At lower exposure concentrations, 0.02-0.06 mg/ℓ (20-60 mg/m^3), weight gain was reduced by 5-10% and blood pressures were slightly elevated, but no overt signs of toxicity were noted as compared to controls. Histological examination at the end of 5 months exposure revealed tissue changes similar to those seen following exposure to 170-250 mg/m^3, but of reduced severity (Kremneva and Tolgskaya, 1961).

The effects of lower atmospheric concentrations were determined by Fomin (1966, 1968), who exposed groups of 15 male rats (strain and weight unspecified) to 0.2, 2.0 or 20 mg epichlorohydrin/m^3 continuously for 98 days, followed by a 28-day recovery period. At the highest dose level, the rats were initially excited, but gradually became sluggish and displayed excessive sleepiness. The animals appeared to return to normal by the end of the third week of exposure. Weight gain in this group was 14-19% less than that of the controls over the 98 day exposure period. Total food (milk) consumption was unaffected; however, a dose-related increase in feeding time was observed during the first 1.5 months of the study. A transient increase in reaction time was noted in the high-dose group between 1.5 and 3 months. At 2 and 20 mg/m^3, decreases in blood nucleic acids, increased number of leucocytes with altered fluorescence, and at 20 mg/m^3 only, increased urinary coproporphyrin levels were also observed; however, these values returned to normal during the recovery period. Histological examination revealed emphysema, fine-focus pneumonia, small areas of edema and loosening and swelling of the adventitia of the blood vessels in the lungs of the high-dose group. Myocardial tissue contained intermuscular fine-focus hemorrhages and congestion of the veins. Degeneration of the epithelium was noted in the convoluted tubules of the kidneys. In the central nervous system, severe but unspecified lesions were observed in the neurons of the myelencephalon, cerebellum and Ammon's horn of the brain. No histological lesions were found in animals exposed to 2 or 0.2 mg/m^3.

Quast et al. (1979a) exposed groups of 20 male and 20 female Fisher 344 rats, Sprague-Dawley rats and B6C3F$_1$ mice to atmospheres containing 0, 5, 25 or 50 ppm (0, 19, 95 or 189 mg/m^3) epichlorohydrin 6 hour/day, 5 days/week, for a total of 61 or 62 exposures for males and females, respectively. Rats were 9-11 weeks old and mice 7-9 weeks old when exposure commenced. After 30 exposures, half of the animals in each group were sacrificed. Clinical studies conducted at the time of sacrifice included urinalysis (rat only), blood urea nitrogen, hematology, serum glucose concentration, serum glutamicpyruvic transaminase activity, serum glutamic-oxaloacetic transaminase activity and serum alkaline phosphatase activity.

Treatment-related conjunctival redness and eyelid spasms without evidence of ocular involvement were observed in rats during the exposure periods. Recovery was rapid between treatments. Reduced activity was noted in the rats in the high- and mid-dose groups during the first 10 days of exposure.

At an exposure concentration of 189 mg/m^3, body weight gain was decreased slightly in male rats and in both sexes of mice. The nasal cavities were severely affected, displaying inflammatory and degenerative changes in the olfactory and respiratory epithelia. Strain and sex differences were observed in rats, with males being more sensitive than females and Sprague-Dawley rats more sensitive than Fisher 344 rats. The mice were less sensitive than either strain of rat, and there were no sex-related differences in sensitivity among mice. Major histologic changes were also observed in the kidneys of the rats, but not mice, in the high-dose groups. The tubules tended to be dilated, and focal tubular nephrosis and swelling of epithelial cells in the renal cortex were common. Similar degrees of damage were noted after either 30 or 61-62 exposures. Hepatic glycogen deposits were depleted in rats, but not in mice. Slight microvacuolation of cells in the zona fasciculata of the adrenal glands was noted in both strains of male rats. The epididymia of male Sprague-Dawley rats contained increased numbers of nucleated cells and amorphous eosinophilic staining material within the lumen.

At an exposure level of 95 mg/m^3, these changes in the nasal cavities and respiratory tract were similar to, but less severe than, those seen following exposure to 189 mg/m^3. No histological lesions were found in any other organ. Exposure to 19 mg/m^3 did not produce measurable effects in any exposure group (Quast et al., 1979a).

Laskin et al. (1980) exposed 140 male Sprague-Dawley rats (8 weeks old at start of treatment) to 100 ppm (378 mg/m^3) epichlorohydrin, 6 hours per day, for 30 days. Observations were continued for the lifetime of the animals. At necropsy, inflammation, accompanied by suppuration and destruction of the mucous membrane of the nasal tubinates, was observed. Inflammatory changes were seen in the larynx and trachea, with increasing frequency from the distal to proximal portions. Lungs were edemic with congestion and pneumonia. Renal damage, consisting of dilation of cortical and medullary tubules and filling with hyaline casts, was observed in 63% of the rats.

In a longer-term study, Laskin et al. (1980) exposed groups of 100 male Sprague-Dawley rats (8 weeks old at start of treatment) to atmospheres containing 0, 10 or 30 ppm (0, 38 or 114 mg/m^3) epichlorohydrin, 6 hours/day, 5 days/week, for the lifetime of the rats. The study also included 50 untreated rats. Severe inflammatory changes were seen in the nasal cavity of 90% of the control rats; thus, the effect of these levels of epichlorohydrin on the nasal turbinates could not be assessed. Some exposed rats (incidence not reported) developed squamous metaplasia of the nasal mucosa, while this lesion was not observed in any of the control animals. Pronounced weight loss was observed in the high-dose group after 40 weeks. Initially, mortality was higher in the control groups than in the epichlorohydrin treated groups, but mortality was equivalent to controls by 48 weeks in the 38 mg/m^3 exposure group and 60 weeks in the 114 mg/m^3 exposure group. Mortality reached 100% in all groups by 136 weeks. Severe lung congestion and pneumonia were found in all exposed groups. Necropsy findings in the lungs of control rats were not reported.

Kidney lesions were found in 65, 37, 24 and 14% of the 30 ppm, 10 ppm, sham control and untreated control rats, respectively. The predominant lesions involved tubular dilation and degeneration of the tubules. The tubular epithelium was flattened and some tubules were filled with hyaline casts. Atrophy and fatty vacuoles in the basal part of the tubular epithelium and atrophy of the glomeruli with hyaline cast replacement were occasionally observed. Lifetime exposure to 114 mg/m^3 produced damage equivalent to 30 exposures to 378 mg/m^3. In animals exposed to 38 mg/m^3, damage was intermediate between that caused by the 114 mg/m^3 level and that of controls (Laskin et al., 1980).

John et al. (1983) exposed groups of 10 male New Zealand rabbits ~6 months old, 30 male (350-450 g) and 30 female (225-300 g) Sprague-Dawley rats to atmospheres containing 0, 5, 25 or 50 ppm (0, 19, 95 or 189 mg/m^3) epichlorohydrin, 6 hours/day, 5 days/week, for 10 weeks, followed by a 10-week recovery period. No deaths occurred in male rats, while three deaths, one each in the control, 19 and 189 mg/m^3 group, occurred in female rats. Three to five animals from each group were sacrificed at the end of the 10-week exposure period for histological examination. After 10 weeks of exposure, kidney weights and kidney-to-body weight ratios were significantly increased (data not given) in both male and female rats exposed to 189 mg/m^3, while body weight gain was significantly decreased (74 and 46 g for males and females in the exposed groups as compared to 92 and 52 g in the respective controls) in this group. Occasional dilated renal tubules and eosinophilic staining casts were observed in both sexes of rats exposed to either 95 or 189 mg epichlorohydrin/m^3. These effects were apparently reversable and were not reported after the 10-week recovery period. Degenerative changes, characterized by inflammation, focal erosions, hyperplasia and metaplasia, were observed in the nasal turbinates of all rats examined at the end of the exposure period. These effects were not detected following the 10-week recovery period.

Epichlorohydrin appeared to be more toxic to rabbits than to rats in this study. In the high-exposure group, weight gain was significantly depressed (-0.13 kg as compared to +0.15 kg in the controls) and 2 of the 10 rabbits died or were killed in moribund condition. Necropsy revealed suppurative rhinitis accompanied by diffuse pneumonia or pleuritis. No histopathological lesions were found in the kidneys; however, dose-related inflammation and erosion of the nasal turbinates, which regressed by the end of the 10-week recovery period, were observed at the end of the exposure period in rabbits exposed to 95 or 189 mg epichlorohydrin/m^3 (John et al., 1983).

Other Routes of Exposure -- Lawrence et al. (1972, 1974) have investigated the toxicity of epichlorohydrin following intraperitoneal administration in cottonseed oil. Groups of 12 male Sprague-Dawley rats (100-150 g) were administered 30 daily intraperitoneal injections of 0.01910 or 0.00955 mℓ epichlorohydrin/kg bw. A vehicle control group was included. At the end of the study, the adrenals, brains, gonads, hearts, kidneys, livers, lungs and spleens were excised for histological examination. Both doses produced significant ($p<0.05$) reductions in weight gain during the course of the experiment. The body weight gains at termination were 183, 167 and 158 for the control, low- and high-dose groups, respectively. Liver function, as determined by the disappearance of sodium sulfobromophthalein, was not affected by this treatment. Hemoglobin was increased ($p<0.05$) in the low-dose group (17.2 g/100 mℓ) but decreased in the high-dose group (14.6 g/100 mℓ) as compared to control levels of 16.3 g/100 mℓ. There was a significant increase in the number of neutrophilic metamyelocytes in the high-dose group. The kidney-to-body weight ratio was significantly ($p<0.05$) increased in both dose groups, but no significant kidney damage was detected on histological examination. Bronchitis, peribronchitis, interstitial pneumonia, bronchopneumonia and emphysema were found in all groups, including controls, but the incidence and severity of these lesions was increased by epichlorohydrin treatment.

In another study, these same authors administered in cottonseed oil 0, 0.0095, 0.0190 or 0.04774 mℓ epichlorohydrin/kg bw to groups of 12 male Sprague-Dawley rats on Monday, Wednesday and Friday for 12 weeks. Weight gain was significantly reduced ($p<0.01$) in the high-dose group (328 g in treated vs. 358 in control group). The hemoglobin level was significantly ($p=0.05$) decreased from 16.5 to 15.5, 15.2 and 11.8 g/100 mℓ in the control, low-, mid- and high-dose group, respectively. In the group receiving 0.04774 mℓ/kg bw, the heart, kidney and liver-to-body weight ratios were increased and brain-to-body weight ratios were decreased. Mortality was 25% (3/12) in the high-dose group, but no deaths occurred at lower doses.

Target Site Toxicity

Nasal Turbinates. The nasal cavities are major targets for inhalation, but not oral, exposure to epichlorohydrin. This is consistent with the toxicokinetic studies in which high levels of epichlorohydrin were deposited in the nasal turbinates following inhalation, but not oral, exposures (see Chapter II, Distribution Section). Damage to these tissues consists of inflammation of the nasal cavities accompanied by degeneration and necrosis of the nasal mucosa (see Dermal Toxicity and Inhalation Toxicity Sections). These effects are reversible once the animals are removed from exposure. Neoplastic lesions of the nasal cavity have also been reported during chronic inhalation exposure, as detailed in Inhalation Section.

Lungs. Pathological changes in the lungs have been reported following both subcutaneous and inhalation exposures. Rotaru and Pallade (1966) and Pallade et al. (1967) administered, by subcutaneous injection, 150 or 180 mg epichlorohydrin to groups of 23 or 14 rats (strain and sex unspecified), respectively. The lungs were found to be congested, with desquamative bronchial inflammation and edema of the bronchiovascular connective tissue.

Inhalation exposure produces a number of severe pathological effects in the lungs. At 4-hour exposures above 1147 mg/m^3, lung-to-body weight ratios in rats increased dramatically, due to severe pulmonary edema (Carpenter et al., 1949). When the rats were exposed to atmospheres containing 1071 mg/m^3 or higher for 4 hours, acute respiratory irritation and hemorrhage were observed. Other investigators have reported that exposure to concentrations as low as 50 ppm (189 mg/m^3), 6 hours/day, 5 days/week, for 88 days can produce focal subacute pneumonitis in mice, but not in rats (Quast et al., 1979a).

Topical Applications. Epichlorohydrin has been demonstrated to produce a number of pathological changes at the site of application. Lawrence et al. (1972) investigated the effect of epichlorohydrin diluted with cottonseed oil on the rabbit eye. In New Zealand rabbits, 0.1 mℓ of various concentrations of epichlorohydrin was applied to the superior temporal quadrant of the right eye, and the eyes were examined every 30 minutes for 3 hours. The 10% concentration produced questionable evidence of irritation. A 20 and 40% solution produced conjunctivitis, iritis and palpebral irritation with edema graded as +1 and +2, respectively. Definite corneal damage was produced by an 80% solution. A 5% solution produced no detectable effects.

Epichlorohydrin also produces severe irritation of the skin following dermal exposure in rabbits, rats, mice and guinea pigs (Smyth and Carpenter, 1948; Kremneva and Tolgskaya, 1961; Lawrence et al., 1972; Freuder and Leake, 1941). Following dermal exposure, capillary permeability increases and the skin becomes hyperemic, with the subsequent development of ulcer-like lesions and scabbing. Repeated application produces superficial necrosis of the skin over areas slightly larger than the area of application. These skin lesions heal gradually over a period of 1-1.5 months following a single application (Kremneva and Tolgskaya, 1961).

Kidneys. The kidney is the major target for the systemic toxicity of epichlorohydrin following either oral or inhalation exposures in rats. The major changes occur in the convoluted tubules, which become dilated and filled with eosinophilic staining casts. The epithelial lining of the tubules then becomes necrotic. Regeneration begins within 5 days of a single subcutaneous injection and is essentially complete within 10 days in rats (Rotaru and Pallade, 1966; Pallade et al., 1967). Atrophy of the glomeruli with hyaline cast replacement has been reported to occur in rats, but with lower frequency than damage to the convoluted tubules (Laskin et al., 1980; Rotaru and Pallade, 1966; Kremneva and Tolgskaya, 1961).

Reproductive Organs. Epichlorohydrin and its major metabolite, α-chlorohydrin, have been reported to produce infertility in male rats, rabbits and mice (See Teratogenicity Section). A single high dose of α-chlorohydrin (100 mg/kg bw) results in the formation of spermatoceles or sperm retention cysts in the efferent ducts and caput epididymis (Cooper et al., 1974; Ericsson, 1970). This effectively blocks the passage of testicular sperm to the epididymis, resulting in prolonged, and possibly permanent, infertility.

At lower doses (10 or 20 mg/kg bw/day for 5 days) an immediate and reversible infertility is produced (Jones et al., 1969). This type of infertility is apparently due to inhibition of glycolysis in epididymal sperm (Mohri et al., 1975).

Oral doses as low as 7-8 mg α-chlorohydrin/kg bw have been reported to effect the fertility of spermatozoa in the cauda epididymidis of the rat (Turner, 1971). Histological effects are not produced until the dose exceeds 9 mg/kg bw. Turner (1971) conducted a thorough examination of the histopathological changes produced in the testes by a single oral dose of epichlorohydrin or α-chlorohydrin (100 mg/kg bw). Within 24 hours, the ductuli efferentes were dilated at their origin. The conus region of the caput epididymidis was distended with spermatozoa, and beyond this area, the tubules were enlarged and filled with a hyaline mass consisting of detached epithelium and contained spermatozoa. By 1 week following dosing, large granulomata were present at the testicular origin of the ductuli efferentes, and the proximal caput had been cleared of debris, remaining empty and shrunken. After 6 months, the ductuli efferentes appeared as fibro-hyalinized bodies, with the fibrotic process extending into the right testis.

Central Nervous System. Many of the effects observed following exposure to epichlorohydrin are indicative of effects on the central nervous system. Freuder and Leake (1941) reported delirium in rats exposed to atmospheric concentrations of 8,300 or 16,600 ppm (31,411 or 62,822 mg/m³) epichlorohydrin for 30 minutes. Kremneva (1960) found that, following exposure to atmospheres containing 1416-2124 ppm (5359-8038 mg/m³), rats became quiescent, developing cyanosis and muscular relaxation of the extremities and progressing to tail stiffening and fine tremors of the body. Death was attributed to depression of the respiratory center.

In longer-term studies, exposure to 20 mg epichlorohydrin/m³ continuously for 98 days was demonstrated to increase the latency time for defensive unconditioned reflex action (response to electrical shock) in rats (Fomin, 1966, 1968). No effect was seen at a concentration of 2.0 mg/m³. At the high exposure level, unspecified histological changes were observed in the medulla oblongata, cerebellum and hippocampus.

Other Effects

Carcinogenicity.

Oral -- Konishi et al. (1980) and Kawabata (1981) reported on a study of the carcinogenicity of epichlorohydrin. Male Wistar rats (18 per group) were administered 0, 375, 750 or 1500 ppm (0, 375, 500 or 1500 mg/ℓ) epichlorohydrin in their drinking water for 81 weeks. Water consumption was measured weekly. After 60 weeks of dosing, epichlorohydrin exposure was intermittently interrupted, due to toxicity. The total dose for the experiment was 0 g/rat, 5.0 g/rat, 8.9 g/rat and 15.1 g/rat, respectively. The results of this study are summarized in Table V-7. The most consistent finding was a dose-related incidence of forestomach tumors. Histologically, these tumors were found to be squamous and basal hyperplasias, squamous cell papillomas or carcinomas. Animals that died before final sacrifice at 81

TABLE V-7

- Number of Forestomach Tumors and Tumor Incidence in Male Wistar Rats Given Epichlorohydrin*

Dose (mg/ℓ)	No. of rats		No. of tumors in forestomach per rat				Histological findings on squamous epithelium (%)		
				Tumor size (mm)					
	Initial	Effective	Total	5<	5>2<	2>	Hyperplasia	Papilloma	Carcinoma
0	18	10	0	0	0	0	0 (0)	0 (0)	0 (0)
375	18	9	5.6±8.4	0	0.1±0.3	5.4±8.2	7 (77.8)	0 (0)	0 (0)
750	18	10	9.9±12.8	0.4±0.5	1.2±1.2	8.3±12.0	9 (90.0)	1 (10.0)	1 (10.0)
1500	18	12	32.8±24.0	0.8±1.0	4.4±3.6	27.6±21.3	12 (100.0)	7 (58.3)	2 (16.7)

*Source: adapted from Konishi et al., 1980 weeks were not examined for pathology. Other tumor types observed in this study included interstitial cell tumors of the testis and squamous cell carcinomas of the oral cavity. Interstitial cell tumors were observed in all groups in approximately equal numbers. Squamous cell carcinomas of the forestomach were found only in the high-dose group (2/12 rats).

In a report of preliminary results, Van Esch (1981, 1982) described exposing 50 male and 50 female Wistar rats to epichlorohydrin at doses of 0, 2 and 10 mg/kg bw, 5 days/week for 104 weeks. Treatment was by gavage with water as the vehicle. At the time of this report, only the stomach had been examined histologically. In the forestomach, both papillomas and carcinomas were observed, with the incidence of carcinomas for females being 0/50, 2/50 and 24/50, and for males, 0/50, 6/50 and 35/50 for the control, low- and high-dose groups, respectively. Additonally, papilloma incidence was 2, 2 and 0 for females and 1, 6 and 2 for males in the control, low- and high-dose groups, respectively.

Inhalation -- Inhalation exposure to epichlorohydrin has been reported to produce respiratory tract tumors. Laskin et al. (1980) exposed 140 male Sprague-Dawley rats to atmospheres containing 100 ppm (378 mg/m^3) epichlorohydrin 6 hours/day, 5 days/week, for a total of 30 exposures. Two control groups, untreated controls and air-treated controls, were included in the study. Mortality was slightly higher in the air-treated controls, due to respiratory infection, than in the other groups. Overall, mortality in the treated animals was equivalent to that in the untreated controls, though the initial mortality (9% in the first 8 weeks) was slightly elevated. Neoplastic lesions developed in 17/140 rats in the nasal cavity between 330 and 933 days following the beginning of exposure. The majority of these tumors (15) were squamous cell carcinomas, which formed a solid mass filling the nasal cavity. There were also 2 nasal papillomas. Histological examination revealed welldifferentiated squamous cell carcinomas with keratin pearls, which appeared to arise from the mucosa of the anterior half of the nasal cavity. Metastasis was not observed; however, many of the squamous cell carcinomas had infiltrated and penetrated into the bones of the skull. No other treatment-related tumors were detected.

Laskin et al. (1980) also exposed groups of 100 male Sprague-Dawley rats to 10 or 30 ppm (38 or 114 mg/m^3) for the lifetime of the animals. Mortality reached 100% by 136 weeks. In the group exposed to 114 mg/m^3, one rat died with a bronchial papilloma at 402 days and one with a squamous cell carcinoma at 752 days. No nasal tumors were found in the group exposed to 38 mg/m^3 or in any other organ at either dose level. In these studies, exposure to high concentrations of epichlorohydrin for short periods of time resulted in a much higher tumor incidence than did long-term exposures to lower concentrations (Table V-8).

Dermal -- The dermal carcinogenicity of epichlorohydrin has been investigated by two groups (Weil et al., 1963; Van Duuren et al., 1974). Weil et al. (1968) clipped the hair from the dorsal skin of 40 90-day-old C3H mice (sex unspecified) and applied a "brushfull" of epichlorohydrin to the midline of the back on Monday, Wednesday and Friday for the lifetime of the animals. Thirty-seven mice were alive after 12 months of treatment, 30 after 17 months and 1 after 24 months; none survived more than 25 months of treatment. No mice developed tumors at the site of application. No necropsy data were presented.

TABLE V-8

Effect of Dose Rate Upon Inhalation Carcinogenesis by Epichlorohydrin[a]

Concentration ppm	No. of exposures	Total dose, ppm-days	Cancer incidence[b]
100	30	3000	15/140[c]
30	290[d]	8700	1/100[e]
10	250[d]	2500	0/100

[a] Source: adapted from Laskin et al., 1980

[b] Values represent number of rats with cancer/total number exposed to epichlorohydrin.

[c] In this group, 1 bronchial and 2 nasal cavity papillomas were also seen, as described in the text.

[d] Lifetime exposures were based upon median survival time.

[e] A nasal papilloma occurring after 634 days was also observed in this group.

Van Duuren et al. (1974) applied 2 mg epichlorohydrin in 0.1 mℓ acetone to the shaved interscapular region of 50 female ICR/Ha Swiss mice 3 times/week for the lifetime of the animals. The median survival time was 506 days and the last mouse died at 580 days. No papillomas or carcinomas were detected on any mouse.

In an initiation-promotion experiment, Van Duuren et al. (1974) applied a single dose of 2.0 mg epichlorohydrin in 0.1 mℓ acetone to the shaved skin of 30 female ICR/Ha Swiss mice. Following 2 weeks of no treatment, phorbol myristate acetate (25 μg in 0.1 mℓ) was applied 3 times/week for the remainder of the study (385 days). The median survival time for this group of mice was greater than the length of the experiment. This treatment resulted in the production of papillomas on nine mice and a carcinoma on one mouse at the site of application. No production of tumors at sites distant from the point of application was reported in either of the Van Duuren et al. experiments.

Subcutaneous and Intraperitoneal Exposure -- Van Duuren et al. (1972, 1974) investigated the carcinogenicity of subcutaneously administered epichlorohydrin. Fifty female ICR/Ha Swiss mice were injected in the left flank with 1.0 mg epichlorohydrin in 0.05 mℓ tricaprylin weekly for 580 days. The mice in this group had a median survival time of 486 days. This treatment produced local sarcomas in 6/50 mice and local adenocarcinomas in 1/50 mice. This represents a significant increase ($p<0.05$) in the incidence of local malignant tumors in mice given weekly subcutaneous injections of epichlorohydrin.

Van Duuren et al. (1974) also investigated the carcinogenicity of epichlorohydrin following intraperitoneal injection. Thirty female ICR/Ha Swiss mice were given weekly injections of 1.0 mg epichlorohydrin in 0.05 mℓ tricaprylin. The experiment was terminated at 450 days. No tumors were produced at the site of injection. Papillary tumors of the lung were found in 11/30 treated mice, however, this was not significantly different from the incidence in mice that were untreated (29/100) or that received 0.05 mℓ tricaprylin/week (10/30). No other tumors were reported at sites distant from the point of injection.

Mutagenicity.

Prokaryotic Systems -- The mutagenicity of epichlorohydrin in prokaryotic systems is summarized in Table V-9. The list of references cited in this table is representative of the extensive literature available on the mutagenicity of epichlorohyrin. Epichlorohydrin is commonly acknowledged to be a potent inducer of base-pair substitution-type mutation and has been used as a positive control in recent publications (e.g., Andersen et al., 1978). The data on the mutagenicity of epichlorohydrin have been summarized by Fishbein (1976) and Sram et al. (1981).

The addition of liver homogenates to the incubation mixture has consistently resulted in a marked reduction in mutation frequency (Table V-9). Kilian et al. (1978) found that the mutagenic response was reduced even if the liver homogenate was heat inactivated, suggesting that the decrease in mutation frequencies was not due to metabolism of the epichlorohydrin. This conclusion contrasts with that of Rossi et al. (1983b), who found that both S-9 mix and purified microsomes, but not cytosol, would reduce the mutagenic

TABLE V-9

Mutagenicity of Epichlorohydrin in Prokaryotic Systems

Indicator Organisms	Strains	Assay	Dose	Metabolic Activating Systems	Response	Comments	Reference
Salmonella typhimurium	NR	NR	NR	-	+	NC	Mukai and Hawryluk, 1973
Salmonella typhimurium	TA100, TA1535	plate incorporation	0.254-25.4 μmol/plate	+/-	+	Mutagenic response was reduced by the addition of S-9 mix.	Andersen et al., 1978
Salmonella typhimurium	TA1535	NR	NR	+/-	+	Mutagenic response was reduced by the addition of S-9 mix, even if the enzyme was heat inactivated.	Kilian et al., 1978
Salmonella typhimurium	TA100, TA1535, TA1537, TA98	plate incorporation	NR	-	+	Positive results in TA100 and TA1535, but negative in TA98 and TA1537 which are sensitive to frame-shift mutagens.	Wade et al., 1978
Salmonella typhimurium	G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, TA98	plate incorporation (modified to produce gradients)	0.1-1000/ μg/mℓ	+/-	+	Positive results in strains sensitive to base substitution mutagens.	McMahon et al., 1979
Salmonella typhimurium	TA100	liquid suspension	0.5-7.5 μmol/ mℓ	+/-	+	Mutagenic response was reduced by the addition of S-9 mix.	Eder et al., 1980
Salmonella typhimurium	G46, TA1535, TA1538, TA98, TA1530, TA100	plate incorporation	200 μmol/plate	+/-		NC	Bartsch et al., 1980
Salmonella typhimurium	TA100, TA1535, G46	Gas, plate incorporation, liquid suspension	0-5 ng/mℓ air, 0.2 μg/mℓ, agar 100-1000 μg/mℓ medium	-	+	Air concentration was calculated by assuming complete volatization of the test compound. Positive results were obtained in all assays.	Bridges, 1978

TABLE V-9 (cont.)

Indicator Organisms	Strains	Assay	Dose	Metabolic Activating Systems	Response	Comments	Reference
Escherichia coli	WP2, WP2uvrA⁻ CM881, CM891	plate incorporation (modified to produce gradients)	0.1-1000 µg/mℓ	+/-	+	Indicates susceptibility to base-pair substitutions.	McMahon et al., 1979
Escherichia coli	B/r	liquid suspension	0.5 molar	-	+	NC	Strauss and Okubo, 1960
Escherichia coli	K12/343/113	liquid suspension	0-13 mmolar	-	+	NC	Mohn et al., 1981
Bacillus subtillis	NR	Rec	NR	-	+	NC	Kanada and Uyeta, 1978
Klebsiella pneumoniae	NR	fluctuation test	0.1-1 mmol/ℓ	+/-	+	The mutagenicity of epichlorohydrin almost totally disappeared when S-9 was added. Enhanced mutation rates were observed at concentrations of 0.2-1 mmole/ℓ.	Voogd, 1973; Voogd et al., 1981
Schizosaccharomyces pombe	P1	liquid suspension	0, 0.8 or 3.2 mM	+/-	+	S-9 but not cytosol reduced mutagenic potency. The authors hypothesize that microsomal epoxide hydrolases detoxify epichlorohydrin.	Rossi et al., 1983b
Schizosaccharomyces pombe	P1	liquid suspension	0-6.4 mM	+/-	+	Mutagenic potency was reduced by S-9.	Migliore et al., 1982
Neurospora conidia	W. 40 "distinetus" A	liquid suspension	0.15 M	-	+	Mutagenic frequency was directly related to length of exposure.	Kolmark and Giles, 1955

NC = No comment

NR = Not reported

potency of epichlorohydrin. They also reported that "the various protein contents of the subcellular fractions were not involved in any scavenger effect as regards ECH mutagenic activity." The addition of reduced glutathione to the reaction mixtures did not significantly affect the mutation frequencies. The authors concluded that microsomal epoxide hydrolases represented the major steps in the detoxification of epichlorohydrin.

Eukaryotic Cell Systems --

Human Lymphocytes.

In Vitro -- Kucerova et al. (1977) and Sram et al. (1976) investigated the effect of 10^{-4} to 10^{-11} molar epichlorohydrin on human lymphocytes *in vitro*. Lymphocytes were cultured for a total of 56 hours and exposed to epichlorohydrin at various times during cultivation. They concluded that the best results were obtained when epichlorohydrin was added for the last 24 hours. At concentrations of 10^{-5}, 10^{-6} and 10^{-7} molar, epichlorohydrin treatment resulted in an increased number of aberrations and an increased number of chromosomal breaks. Under the conditions of this assay, the effect of epichlorohydrin was 4-5 times lower than that of the polyfunctional alkylating agent, TEPA.

Epichlorohydrin has also been demonstrated to increase SCE in cultured human lymphocytes (White, 1980). Human lymphocytes were cultured in the presence of 5-bromo-2'-deoxyuridine for 73 hours. The addition of epichlorohydrin for either the entire incubation period or the last 25 hours only produced similar levels of SCE, however, tolerance was increased in the latter case. Under these conditions, a concentration of 4×10^{-4} M epichlorohydrin produced a 3-fold increase in SCEs. When a rat-liver S-9 mix was added to the incubation, a 10-fold increase in epichlorohydrin concentration was required to produce similar levels of SCEs.

At still lower concentrations of 1×10^{-5} and 1×10^{-8} M epichlorohydrin, Carbone et al. (1981) observed a dose related increase in SCE in human lymphocyte cultures from three healthy female volunteers. The epichlorohydrin was added 52 hours after the preparation of the cultures and exposure was continued for 20 hours. The lowest dose tested, 1×10^{-11} M epichlorohydrin failed to produce a significant increase in SCE.

Norppa et al. (1981) have investigated the production of both chromosome abnormalities and SCE by epichlorohydrin. For analysis of chromosomal aberrations, cultures were incubated for 24 hours before treatment and then exposed to 0.05, 0.2 or 0.4 mM epichlorohydrin for 24 hours (48-hour cultures). Conditions were the same for studies in which the production of SCEs was the observed endpoint, except that the culture media contained 5 μg bromodeoxyuridine and exposure to epichlorohydrin lasted for 48 hours (72-hour cultures). The results of this study are summarized in Table V-10. At a concentration of 0.05 mM, epichlorohydrin induced SCEs, but not chromosome aberrations. A concentration of 0.20 mM was sufficient to induce chromosome aberrations if the exposure lasted 48 hours.

Dean and Hodson-Walker (1979) investigated the DNA-damaging capacity of epichlorohydrin in a rat-liver derived cell line (RL_1). Cultures were exposed to 0, 5, 10, 15 or 20 μg epichlorohydrin/mℓ of culture media and scored for chromosome aberrations, chromatid gaps, chromatid deletions and

TABLE V-10

Chromosome Aberrations in PHA-Stimulated Human Lymphocytes[a]

Treatment			Cells with Aberrations							
			Chromatid Type					Total		
mM	(µg/mℓ)	Number Cells Studied	Breaks	Exchanges	Total	Chromosome-Type Breaks	Gaps	Gaps Excluded	Gaps Included	Mean Number of SCEs/Cell Standard Error (50 cells Analyzed/Treatment)
Control, acetone										
12.8	(741.4)	200	1.0	0.0	1.0	0.5	0.5	1.5	2.0	7.1±0.4
Epichlorohydrin 24 Hours After Treatment (48-hour Cultures)										
0.05	(4.6)	200	0.5	0.0	0.5	0.0	1.5	0.5	2.0	NA[b]
0.20	(18.5)	200	2.0	0.0	2.0	0.0	4.0^{c}	2.0	6.0^{c}	NA
0.40	(37.0)			No metaphases						NA
Epichlorohydrin 48 Hours After Treatment (72-hour Cultures)										
0.05		100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.4±0.4^{c}
0.20		100	11.1^{d}	2.8^{c}	13.9^{d}	2.8^{c}	2.8	16.7^{d}	19.5^{d}	30.5±1.2^{d}
0.40		100	15.6^{d}	6.4^{c}	22.0^{d}	2.7^{c}	3.8	24.7^{d}	28.5^{d}	56.3±2.0^{b,d}

[a] Source: Adapted from Norppa et al., 1981

[b] 19 Metaphases analyzed

[c] $p<0.05$

[d] $p<0.01$, Fishers exact probability test (one-tailed)

NA = Not analyzed

chromatid exchanges. The frequency of chromatid gaps increased in a dose-dependent manner at concentrations of 10 μg/mℓ or above. The only other aberration observed was an increase in chromatid exchanges at the highest concentrations.

In Vivo -- A number of investigators have studied the effect of occupational exposures to epichlorohydrin on the frequency of chromosomal aberrations in human lymphocytes. Kucerova et al. (1977) conducted a prospective cytogenic study in 35 workers in Czechoslovakia. The workers in this study ranged in age from 23-54 years, and had no known history of exposure to radiation or chemical mutagens. Blood samples were obtained from employees before the start of employment, and after 1 and 2 years of employment in an epichlorohydrin manufacturing plant. For each blood sample, cells were cultivated for 56-58 hours, four slides were prepared for each sampled worker, and ~50 cells were scored on each slide. Exposures ranged from 0.5-5.0 mg/m³, averaging above the maximal acceptable concentration [1 mg/m³ (Sram, 1980)] recommended for epichlorohydrin in Czechoslovakia. On the initial testing, at the beginning of the workers' employment, the average percentage of aberrant cells was 1.37%. After 1 year of employment, this value had increased to an average 1.91% ($p=0.025$) and to an average 2.69% ($p<0.0001$) after 2 years.

Similar results have been reported by Picciano (1979a). In this study, peripheral lymphocytes were obtained from 93 workers employed in two separate (unspecified) facilities. The control group consisted of 75 preemployment individuals matched to the exposed workers for sex and the time period during which the lymphocytes were cultured. The exposed workers ranged in age from 20-62 years (35.8 years average), while the preemployment group ranged from 20-49 year (25.2 years average). Cells were cultured for 72 hours and 200 metaphase spreads were analyzed per individual. No exposure data were available for the workers in this study. The results are summarized in Table V-11. There were statistically significant changes in the number of total abnormal cells, severely damaged cells, chromosome breaks and chromatid breaks and in the distribution of these aberrations within groups. In a follow-up study, Picciano (1979b) repeated these cytogenetic studies in the 17 workers who had the highest levels of chromosome damage in the earlier study. The control population consisted of 18 nonexposed individuals. Five months elapsed between studies, and efforts were made to clean the plant and to reduce exposures during this time. The frequency of abnormal cells in the exposed population was reduced compared to the original analysis, but there were still significantly more chromatid breaks in the exposed group than in the control ($p<0.05$).

Sram et al. (1980) studied chromosomal aberrations in 28 workers who were exposed for a total of 4 years to epichlorohydrin concentrations, and in excess of 1 mg/m^3 for at least 2 years prior to analysis. Control groups consisted of 34 matched controls and 21 subjects from the general population, none of which had a prior history of exposure to radiation or other mutagenic agents. The frequency of aberrations in the 28 workers (3.12% for the 6,306 cells analysed) was significantly greater than the frequency in the matched controls (2.06%, $p<0.01$ for 3400 cells) or the general population sample (1.33%, $p<0.01$ for 2100 cells). Scoring data were also given as a total of 5206 cells from 23 exposed subjects which had been reviewed by Kucerova et al. (1977). Aberration frequency for the 23 workers

TABLE V-11

Cytogenetic Summary of 93 Epichlorohydrin Workers and 75 Nonexposed Individuals[a]

Group	Number in Group	Percent of Individuals with Abnormal Cells			
		0	1-6	7-12	>12
Nonexposed	75	12.0	57.3	26.7	4.0
Exposed	93	2.2	51.6	24.7	21.5
		Percent of Individuals with Chromosome Breaks			
		0	1-2	3-4	>4
Nonexposed	75	53.3	30.7	14.7	1.3
Exposed	93	26.9	50.5	10.8	11.8
		Percent of Individuals with Chromatid Breaks			
		0	1-6	7-12	>12
Nonexposed	75	17.3	60.0	17.3	5.3
Exposed	93	5.4	52.7	21.5	20.4

	Number of Observations	
	Nonexposed Individuals	Exposed Workers
Individuals	75	93
Cells	15,000 (100%)	18,600 (100%)
Chromatid breaks	323 (2.15%)	807 (4.34%)
Chromosome breaks	77 (0.51%)	179 (0.96%)
Marker chromosomes[b]	12 (0.08%)	24 (0.13%)
Severely damaged cells	2 (0.01%)	23 (0.12%)
Abnormal cells	357 (2.38%)	791 (4.25%)

[a] Source: Adapted from Picciano, 1979a

[b] Rings, dicentrics and translocations

given in the data by Sram et al. (1980) was 3.02%. In a related paper, Sram (1980) postulates that "what was originally believed to be the effect of occupational exposure to epichlorohydrin could, in fact, had resulted from a changing pollution pattern in the town where the plant is situated," but the basis for this conclusion was not evident.

In a Dow Chemical Company study (Dow, 1979), apparently using the same data base as Picciano (1979a), it was concluded that "these studies cannot be used to support the theory that groups with occupational exposure to epichlorohydrin will sustain increased chromosomal aberrations as a result of the exposure." In this analysis, the exposed group was reduced from 93 to 76 individuals and the control group from 75 to 26. This reduction was based upon employment history, the time of year in which the blood was drawn and prior exposure to other industrial chemicals. The initial statistical analysis indicated an increase in cytogenetic aberrations in the exposed group ($p=0.03$); however, this difference was found to be due largely to the differences in the growth media used at different times. When the data were reanalyzed adjusting for differences in media, no significant differences were found between exposed and control groups ($p=0.31$).

Mammalian Cell Cultures. Moore-Brown and Clive (1979) treated mouse lymphoma L5178Y/TK$^{+\ -}$ cells with 20, 40 or 60 μg epichlorohydrin/mℓ. Two types of mutations were produced at the thymidinekinase locus. The production of large mutant colonies followed a linear dose-response curve that was considered to be typical of a one-hit point mutational mechanism. The dose-response curve for the production of smaller mutant colonies was, however, not linear and suggested a multi-hit mechanism for the induction of mutations.

Tests in *Drosophila* -- The earliest reported test of epichlorohydrin mutagenicity in *Drosophila* was that of Rapoport (1948). The concentrations used, route of administration and other details of the experimental design were not presented. The frequency of sex-linked recessive lethal mutations was reported to be 0.7% in the treated groups, compared to 0% in the control group.

Two studies have found that epichlorohydrin is not mutagenic to *Drosophila* when it is incorporated in the food. Wurgler and Graf (1981) dissolved epichlorohydrin in 2% dimethylsulfoxide and diluted this solution in a buffered 5% sucrose solution (pH 6.8). This resulted in a 0.2% epichlorohydrin solution which was fed to 2-day old male Berlin-K flies for 3 days on glass filters. A total of 2209 chromosomes were tested, resulting in 7 (0.32%) recessive lethals. Recessive lethal frequencies were 0.28% in water-treated controls and 0.40% in solvent-treated controls. Similar results were obtained by Knapp et al. (1982). Male Oregon-K flies, 4 days old, were fed solutions containing 2.6, 5.1 or 25.5 mM epichlorohydrin for 24 hours. The two highest concentrations were toxic, resulting in 70 and 100% mortality, respectively. No sex-linked recessive lethals were detected in flies fed 2.6 and 5.1 mM epichlorohydrin. Each male was mated to two virgin females at 2-day intervals following treatment for a total of 4-5 matings. This concentration induced sex-linked recessive lethals in 2 of 5 broods studied when injected, as detailed below.

Positive results are obtained when the epichlorohydrin is administered by injection. Vogel et al. (1981) reported that sex-linked recessive lethals were induced in *Drosophila* when epichlorohydrin was administered by injection, but details of the study were not presented.

Knapp et al. (1982) injected 4-day-old male Oregon-K flies with 2.6, 5.1 or 25.5 μmol of epichlorohydrin. After treatment, males were mated every 2 days to produce a total of five broods, each from three individually mated Basc females, which were evalulated at each dose level. A dose of 2.6 μmol produced 0.93 and 0.19% sex-linked recessive lethals in 2/5 broods, containing 537 and 521 chromosomes, respectively. Higher doses resulted in sex-linked recessive lethals in all broods, with average frequencies of 0.37% (0.10-0.52%) in broods from flies receiving 5.1 μmol epichlorohydrin and 0.72% (0.29-1.04%) in broods from flies injected with 25.5 μmol. Control broods had average recessive lethal frequencies of 0.21% (0.17-0.34%).

In Vivo Mammalian Assays --

Dominant Lethal Assay. Two groups, Epstein et al. (1972) and Sram et al. (1976), have tested epichlorohydrin in the mouse dominant lethal assay. Epstein et al. (1972), in a screening study of 174 chemicals, administered 150 mg epichlorohydrin/kg bw to 10, 8-10-week-old male ICR/Ha Swiss mice by a single intraperitoneal injection. Each male was then mated with 3, 8- to 10-week-old untreated virgin female mice per week for 8 weeks. The authors reported that the frequencies of early fetal deaths and preimplantation loses were within control limits, but actual counts were not reported.

Sram et al. (1976) administered single intraperitoneal doses of 5, 10 or 20 mg epichlorohydrin/kg bw or oral doses of 20 or 40 mg/kg bw to groups of male ICR mice. Other groups received 1 or 4 mg/kg bw/day for 5 consecutive days by intraperitoneal injection or 4 or 16 mg/kg bw/day orally (reported as 5 or 20 mg/kg bw/day in another section of the paper). Each male was then bred to two virgin females/week for 8 weeks. No changes characteristic of dominant lethality were reported for any of the epichlorohydrin-treated groups as compaired to dimethylsulfoxide-treated controls.

Micronucleus Assay. Epichlorohydrin has also produced only negative results in the mouse micronucleus assay. Kirkhart (1981) administered two consecutive daily intraperitoneal doses of 0.0225, 0.045 or 0.09 mg epichlorohydrin/kg bw to groups of eight male ICR mice. Four animals per group were sacrificed at 6 and 30 hours post-treatment, and femoral marrow smears were analyzed for micronuclei and 1000 polychromatic erythrocytes were examined at each dose level. The frequency of micronuclei at each dose level (1.0, 1.5 and 1.2%) was not statistically different ($p>0.05$) from the negative controls (0.4%).

A negative response in this test was also reported by Tsuchimoto and Matter (1981), who administered two consecutive daily doses 0.02, 0.04 or 0.08 mg epichlorohydrin/kg bw to groups of two male and two female CD-1 mice by intraperitoneal injection. The mice were sacrificed 6 hours after the last dose; femoral bone marrow smears were prepared for analysis, and 1500 polychromatic erythrocytes were analyzed from each animal. Epichlorohydrin was reported to give negative results in this test, but the actual data were not presented.

In Vivo Cytogenetics. Mixed results have been obtained in studies of the clastogenic effects of epichlorohydrin *in vivo*. Sram et al. (1976) administered single intraperitoneal doses of 1, 3, 5, 10, 20 or 50 mg/kg bw, five consecutive daily intraperitoneal injections of 10 mg/kg bw; five intraperitoneal injections of 5, 10 or 20 mg/kg bw over 7 days, single oral doses of 5, 20, 40 or 100 mg/kg bw; five consecutive daily oral doses of 20 mg/kg bw; and five oral doses of 20 mg/kg bw over 7 days to female ICR mice. Sacrifices occurred at 6, 24 and 48 hours after single doses or 6 hours after the final dose in multiple dose regimens. The results of this study are summarized in Table V-12. There were clearly dose-related increases in the frequency of aberrations in these studies, with chromatid breaks being the major aberration obseved. In a review article, Sram et al. (1981) report that doses of 5-20 mg epichlorohydrin/kg bw (route not specified) produced 2.0-2.4% abnormal cells in Chinese hamster bone marrow cells, compared to 0.6% in controls. They cited Sram et al. (1976) as the source of this data, but no chomosomal studies in Chinese hamsters are reported in Sram et al. (1976).

Negative results were reported in similar experiments with CD1 mice (Rossi et al., 1983a). Single oral doses of 50 or 200 mg epichlorohydrin/kg bw were administered, and the mice were sacrificed after 6, 24 or 48 hours. Bone-marrow smears were prepared from the femur and examined for abnormal cells. Epichlorohydrin did not significantly ($p>0.05$) affect the frequency of aberrant cells.

Epichlorohydrin has also failed to produce chromosomal aberrations in rats (Dabney et al., 1979). Groups of 10 male and 10 female rats were exposed to atmospheres containing 0, 5, 25 or 50 ppm (0, 19, 95 or 189 mg/m^3) epichlorohydrin, 6 hours/day, 5 days/week, for 4 weeks. Aberration frequencies were not significantly ($p>0.05$) elevated (0.1-0.4%) in exposed groups as compared to controls.

TABLE V-12

Epichlorohydrin-Induced Changes in Mouse Bone Marrow After Intraperitoneal or Oral Application*

Dose (mg/kg)	Hours	Total Number of Cells	Abnormal Cells (%)	Breaks (%) Cells	B/C	Gaps (%) Cells	G/C
Single i.p. Application							
1	24	250	5.2	2.8	0.033	3.2	0.040
3	24	250	8.4	6.0	0.084	3.2	0.040
5	24	250	10.8	10.0	0.112	3.2	0.032
10	24	250	21.6	16.0	0.360	7.2	0.095
20	24	250	28.4	27.2	0.488	3.2	0.040
50	24	250	27.6	20.4	0.360	9.2	0.124
50	6	250	32.8	30.0	0.560	12.8	0.204
50	48	250	22.0	23.6	0.392	6.4	0.088
Repeated i.p. Doses (interval between doses 24 hours)							
5x10	6	250	35.2	20.8	0.350	12.8	0.170
Repeated i.p. Doses (5 doses per 7 days)							
5x5	6	250	38.0	37.2	0.692	7.2	0.072
5x10	6	250	36.0	33.6	0.600	9.2	0.124
5x20	6	250	80.4	78.8	2.176	5.2	0.080
Control DMSO		500	4.0	0.6	0.006	3.8	0.038
Single Oral Application							
5	24	250	6.0	4.8	0.064	2.0	0.020
20	24	250	24.0	23.2	0.372	3.2	0.036
40	24	250	22.4	17.6	0.288	9.2	0.132
100	24	250	29.5	25.2	0.372	12.4	0.176
100	6	250	30.0	23.2	0.350	12.8	0.175
100	48	250	34.0	32.0	0.648	4.4	0.052
Repeated Oral Doses (interval between doses 24 hours)							
5x20	6	250	30.4	26.0	0.450	12.8	0.185
Repeated Oral Doses (5 doses per 7 days)							
5x20	6	250	36.0	29.2	0.460	12.8	0.184
Control DMSO		500	4.0	0.6	0.006	3.8	0.038

*Source: Adapted from Sram et al., 1976

B/C = Breaks per cell; G/C = Gaps per cell

Teratogenicity. Two groups of researchers have investigated the teratogenic potential of epichlorohydrin (Pilny et al., 1979; Marks et al., 1982). In an unpublished report cited in U.S. EPA (1983), Pilny et al. (1979) investigated the teratogenicity of epichlorohydrin in Sprague-Dawley rats and New Zealand rabbits. In preliminary studies, groups of five to six pregnant rats and five pregnant rabbits were exposed to atmospheres containing 0, 25, 50 and 100 ppm (0, 95, 189 or 378 mg/m^3) epichlorohydrin. The animals were exposed 7 hours/day during gestation days 6-15 (rats) and 6-18 (rabbits). Controls were exposed to filtered air. Exposure to 189 or 378 mg/m^3 resulted in decreased maternal weight gain, decreased maternal intra-abdominal adipose tissue and increased respiratory tract infections. In the high-dose group of rats, one rat had normal appearing fetuses, three had only resorption sites and two had no implantation sites. No indications of fetal toxicity were observed in the rabbits. Using doses which did not cause severe maternal toxicity in the preliminary study (2.5 and 25 ppm; 9.5 and 95 mg/m^3), Pilny et al. (1979) then performed a full-scale teratology study using 43-46 rats and 20-25 rabbits/group. The animals were exposed under the protocol used in the preliminary study. The fetuses were removed from rats and rabbits on gestation day 21 and 29, respectively. Rats exposed to 95 mg epichlorohydrin/m^3 consistently weighed less than the control rats and consumed significantly more water. No maternal toxicity was observed in rabbits. Exposure to either of these concentrations had no effect on the incidence of external skeletal or soft-tissue malformations in either rats or rabbits. In addition, there were no alterations in pregnancy rates, litter size, corpora lutea, implantation sites, resorption sites, number of dead fetuses/litter, fetal body weight or crown-rump length.

Marks et al. (1982) administered daily oral doses of 0, 4, 80 or 160 mg epichlorohydrin in cottonseed oil/kg bw/day to CD rats and 0, 80, 120 or 160 mg/kg bw/day to CD-1 mice on days 6-15 of gestation. The mice were sacrificed on day 18 and the rats on day 21. For rats the numbers of dams alive at day 18 were 35, 14, 24 and 24; for mice, 49, 31, 24 and 29 for the control, low-, median- and high-dose groups, respectively. In the rat study, the two highest doses produced signs of maternal toxicity and a higher dose (240 mg/kg/day) killed 5/5 clams before dosing on day 4. A dose of 80 mg/kg bw/day was the only dose which produced a significant ($p<0.05$) decrease in maternal body weight gain (95±1.97 vs. 84±4.35 g). In the group given 160 mg/kg bw/day, 3/27 dams died and the average liver weight of the survivors was significantly ($p<0.05$) increased over control values (15.0±0.35 vs. 16.4±0.37 g). Maternal toxic effects were also observed in the mouse study, where a dose of 160 mg/kg bw/day was lethal to 3/32 mice and a dose of 120 mg/kg bw/day resulted in a significant ($p<0.05$) increase in maternal liver weight (2.37 vs. 2.52 g). In addition, maternal body weight gain was elevated in the low- and mid-dose groups as compared to controls.

Exposure to epichlorohydrin did not result in an increased incidence of malformed fetuses or a decrease in reproductive parameters in either species at any dose tested. The only significant effect was a significant ($p<0.05$) dose-dependent decrease in average fetal weight in mice administered 120 or 160 mg/kg bw/day (0.991 for controls vs. 0.925 vs. 0.898 g).

Reproduction. A number of studies have indicated that epichlorohydrin interferes with reproductive performance in rats and rabbits. Hahn (1970) administered 12 consecutive oral doses of 15 mg/kg bw/day to male Sprague-

Dawley rats of proven fertility. Neither sexual libido nor ejaculation ability was adversely effected by epichlorohydrin treatments. No histological effects were observed in the testes, epididymides, prostate or seminal vesicles at the end of the treatment period. Epichlorohydrin treatment did, however, produce sterility after 1 week of dosing. The effect was temporary, with fertility returning 1 week after treatment was discontinued.

Cooper et al. (1974) investigated the effects of higher doses of epichlorohydrin on reproductive performance in adult male Wistar rats. Groups of five rats were given doses ranging from 20-100 mg epichlorohydrin in arachis oil/kg bw and then sequentially mated to unexposed females for 10 consecutive weeks. Five consecutive daily doses of 50 mg/kg bw/day resulted in total sterility which was not reversed within 10 weeks. Five consecutive daily doses of 20 mg/kg bw/day also produced sterility; however, in this case the effects were reversible after 3 weeks. A single dose of 100 mg/kg bw did not produce sterility, but fertility was reduced throughout the 10-week observation period. Histologically, these treatments produced lesions in the efferent ducts and large retention cysts in the ductuli efferents and proximal caput (see Reproductive Organs Section).

Van Esch (1981) exposed four female and two male Wistar rats by gavage to epichlorohydrin dissolved in water at dose levels of 0, 5, 10 or 20 mg/kg bw, 5 days/week for 10-15 weeks. The rats were mated 4 days after the first treatment, and the resulting pups observed for 21 days post-parturition. The females in the high-dose group failed to become pregnant, while the fertility index (100%), pup weight (9-10 g at birth), viability index (~96%) and lactation index (86-100%) were similar in the 5 and 10 mg/kg groups.

The results from the control group were not reported in this study. In a follow-up study using a similar exposure schedule, Van Esch (1981) treated 20 female and 10 male rats/group with epichlorohydrin at 0, 2 or 10 mg/kg bw. Treatment was started 10 days prior to mating and continued for ~3 months. In the 10 mg/kg group, the fertility index was reduced to 3.3% as compared to 55% in the controls. In addition, slight decreases in hematologic parameters and body weight were observed in this group. No effects of treatment were observed at 2 mg/kg bw. By cross-mating treated (10 mg/kg group) and control animals, it was determined that epichlorohydrin had adverse effects on male reproductive capacity but not female.

The effect of epichlorohydrin on reproductive performance has also been investigated by John et al. (1979, 1983). Groups of 10 male New Zealand rabbits, 30 male and 30 female Sprague-Dawley rats were exposed to atmospheres containing 0, 5, 25 or 50 ppm (0, 19, 95 or 189 mg/m^3) epichlorohydrin, 6 hours/day, 5 days/week, for 10 weeks.

Exposure to 189 mg epichlorohydrin/m^3 induced signs of toxicity in both rats and rabbits. Weight gain was significant ($p<0.05$) in male (74 vs. 92 g) and female (46 vs. 52 g) rats and male (-0.13 vs. 0.15 kg) rabbits , with two rabbits either dying or being sacrificed due to moribund conditions during the treatment period. A dose-related increase in both kidney weight and kidney-to-body weight ratios was observed in male and female rats exposed to 189 mg/m^3 (data not reported). Histopathological changes were observed in the nasal turbinates of both sexes of rats exposed to 95 or 189 mg epichlorohydrin/m^3 (see Inhalation Toxicity Section).

The fertility of the male rats was evaluated by mating them with two unexposed females for 1 week on weeks 2, 4, 7, 10, 12 and 20 of the study. Exposure to 19 mg/m^3 had no effect on reproductive performance. At the two highest exposure levels, there were significantly fewer implants during the treatment period with 1-4 and 7-9 implants/dam in the 189 and 95 mg/m^3 group as compared to 13-14 implants/dam in the control group, but this effect was not noticeable in the matings performed after the end of the treatment period. The fertility of exposed female rats was evaluated at the end of the treatment period by mating them with two unexposed males on two consecutive 5-day periods.

No decrease in fertility was observed in female rats at any exposure level. Additionally, no changes in other indices of reproduction (length of gestation, litter size, survival indices, sex ratio and incidences of malformations) were observed.

The male rabbits were mated with untreated females at the end of the 10-week exposure period. Ovulation was induced with human chorionic gonadotrophin hormone. Exposure of the males to epichlorohydrin did not produce any dose-related changes in fertility, number of implantation sites, corpora lutea or resorptions. Starting 2 weeks before the beginning of the exposure period, rabbit semen was evaluated weekly for quality and quantity. No alterations were found in semen volume, sperm concentrations, sperm motility or sperm morphology (John et al., 1979, 1983).

Summary

Epichlorohydrin is acutely toxic following oral, percutaneous, subcutaneous or respiratory exposure. Symptoms are similar following any route of exposure and include both local and systemic effects. At the site of application, epichlorohydrin is a strong irritant. When absorbed, the major effects occur in the respiratory and central nervous systems. Death results from the central nervous system effects, which have been described as an initial period of erratic movement, followed by listlessness and reduced respiration (Freuder and Leake, 1941).

The oral LD_{50} of epichlorohydrin is ~250 mg/kg bw in both rats and mice (see Table V-1), though values as low as 90 mg/kg bw have been reported (Smyth and Carpenter, 1948). Values for the LC_{50} following inhalation exposure vary widely, with reported values ranging from 1362 mg/m³ for a 6-hour exposure (Laskin et al., 1980) to 4000 mg/m³ for a 1.5-hour exposure (Kremneva, 1960). Oral doses as low as 10 mg/kg bw for 5 days/week for 2 weeks resulted in decreased erythrocyte counts in male rats, and similar exposures to 40 or 80 mg/kg bw resulted in degenerative changes in the kidney in both male and female rats (Van Esch, 1981).

Dermal exposure results in discoloration and superficial desquamation within a few hours (Freuder and Leake, 1941). With continuing exposure, a superficial necrosis develops and the skin becomes dry, parchment like and brown. These effects are slowly reversible after the application of epichlorohydrin is discontinued.

The pathological changes observed following chronic exposure to epichlorohydrin are qualitatively similar to those observed following acute exposures. The limited data on the effects of orally administered epichlorohydrin indicate that doses as low as 94 mg/kg bw produce 100% mortality in mice within 3 weeks (Freuder and Leake, 1941). Gage (1959) reported a NOEL of 34 mg/m³ for rats exposed to epichlorohydrin vapors 6 hours/day, 5 days/week, for a total of 18 exposures. Other investigators have reported reduced weight gain and elevated blood pressure following chronic exposure to 20 mg epichlorohydrin/m³ (Kremneva and Tolgskaya, 1961).

The major target organs for epichlorohydrin toxicity following oral or inhalation exposure are the nasal tubinates, lungs, kidneys, reproductive organs and central nervous system. Following inhalation, but not oral, exposure, the nasal cavities become inflammed and the nasal mucosa degenerates and becomes necrotic. These effects are reversible once the animals are removed from exposure. Pathological changes in the lungs are characterized by congestion with desquamative bronchial inflammation and edema (Rotaru and Pallade, 1966; Pallade et al., 1967; Carpenter et al., 1949). Kidney damage occurs mainly in the convoluted tubules, which become dilated and filled with eosinophilic staining casts (Rotaru and Pallade, 1966; Pallade et al., 1967). Atrophy of the glomeruli with hyaline cast replacement has been reported to occur, but with lower frequency than damage to the convoluted tubules (Laskin et al., 1980; Kremneva and Tolgskaya, 1961).

Epichlorohydrin and its major metabolite, α-chlorohydrin, can produce infertility in males by two distinct mechanisms. At high doses (~100 mg/kg bw), spermatoceles are formed in the efferent ducts and caput epididymis

(Cooper et al., 1974; Ericsson, 1970) resulting in prolonged, possibly permanent, infertility. At lower doses (10-20 mg/kg bw/day for 5 days) a reversible infertility is induced (Jones et al., 1969), possibly due to the inhibition of glycolysis in epididymal sperm (Mohri et al., 1975).

Death following exposure to epichlorohydrin is generally attributed to central nervous system effects, predominately to depression of the respiratory center (Kremneva, 1960). Unspecified histological changes have been observed in the medulla oblongata, cerebellum and hippocampus following inhalation exposure to concentrations as low as 20 mg epichlorohydrin/m^3 (Fomin, 1966, 1968).

Epichlorohydrin has been found to be carcinogenic following oral (Konishi et al., 1980; Kawabata, 1981), inhalation (Laskin et al., 1980) exposure or by subcutaneous injection (Van Duuren et al., 1972, 1974). Oral exposure produces a dose-dependent increase in forestomach tumors characterized as squamous and basal hyperplasias, squamous cell papillomas or carcinomas. Inhalation exposure produces squamous cell carcinomas in the nasal cavities, many of which infiltrate and penetrate the bones of the skull.

In a number of experiments, epichlorohydrin has failed to produce tumors following dermal application (Weil et al., 1963; Van Duuren et al., 1972, 1974). In an initiation-promotion experiment, however, Van Duuren et al. (1974) found epichlorohydrin to be an initiator in ICR/Ha Swiss mice. Qualitatively, it may be surmised that epichlorohydrin is an initiator and not a good promoter or not a promoter at all (U.S. EPA, 1984).

The carcinogenicity of epichlorohydrin is consistent with its strong mutagenic activity (see Table V-9; reviewed in Fishbein, 1976; Sram et al., 1981). Epichlorohydrin is a potent inducer of base-pair substitution-type mutations in prokaryotic systems. Incubation with mammalian liver homogenates results in a marked reduction in mutation frequency. Epichlorohydrin also induces chromosomal aberrations in mammalial cell cultures (Moore-Brown and Clive, 1979) and in human lymphocytes *in vitro* (Kucerova et al., 1976; Sram et al., 1976; White, 1980; Norppa et al., 1981). Examination of occupationally exposed workers indicates that these aberrations also occur *in vivo* (Kucerova et al., 1977; Picciano, 1979a,b).

In *in vivo* studies, epichlorohydrin treatment results in an increased incidence of sex-linked recessive lethals in *Drosophila* when administered by injection, but not when incorporated in the food (Knapp et al., 1982). In other *in vivo* studies, epichlorohydrin has produced negative results in the mouse dominant lethal assay (Epstein et al., 1972; Sram et al., 1976) and the mouse micronucleus assay (Kirkhart, 1981; Tsuchimoto and Matter, 1981).

Epichlorohydrin has not been demonstrated to be teratogenic following either inhalation or oral exposure (Pilny et al., 1979; Marks et al., 1982), even at doses that produced maternal toxicity. Epichlorohydrin does, however, interfere with fertility in male rats. Temporary sterility has been induced by 12 consecutive daily doses of 15 mg/kg bw/day (Hahn, 1970). At higher doses, 5 consecutive daily doses of 50 mg/kg bw/day, an irreversible sterility is produced (Cooper et al., 1974). When both male and female rats were given a daily dose (5 days/week) of epichlorohydrin at 20 mg/kg, 4 days prior to mating and during the subsequent 9-14 weeks, no offspring were produced (Van Esch, 1981). In the same report, a dose of 10 mg/kg bw started 10 days prior to mating decreased the fertility index. These results reflected adverse effects on male reproductive capacity. Epichlorohydrin does not appear to affect the fertility of female rats (John et al., 1979).

VI. HEALTH EFFECTS IN HUMANS

Clinical Case Studies

Dermal Exposure. One report was located in which the effects of acute human dermal exposure to epichlorohydrin were discussed (Ippen and Mathies, 1970). This report describes the fate of five workers who suffered one or two exposures to epichlorohydrin or a mixture of epichlorohydrin and methanol.

The first case is that of a 25 year old chemical worker who spilled a mixture of methanol and epichlorohydrin on both hands. Redness and burning of the hands developed 2 days later and intensified noticeably by the third day. When he reported to an outpatient clinic, his hands were red and swollen to the wrists, with several small blisters a few millimeters in diameter. This condition responded to treatment with corticoid ointment and Ronicol (a vasodilator), but redness and blisters on the wrists were still present 43 days later.

In another case involving exposure to the hands, a 21 year old male worker wiped up a 4:6 epichlorohydrin mixture (~1.5 ℓ) from a laboratory floor. He reportedly washed his hands thoroughly with soap and water; however, redness and itching of the palms developed. Four days later he began applying a corticoid salve, but the condition worsened and, 2 days later, he was admitted to the hospital with intense itching and red, swollen fingers. He remained in the hospital for 11 days, during which time he was treated with a heparinoid salve and intravenous injections of Reparil, a saponin mixture. The redness and swelling gradually abated, but the surfaces of his hands remained hard and rough.

A third case involves a 29 year old male who accidentally spilled epichlorohydrin on his right pants leg. A burning sensation and redness developed within 10 minutes; however, he applied an anesthetic ointment and continued to work. The redness and burning increased with time, and 62 hours after the exposure, he reported to an outpatient clinic. At this time, there were two large patches of redness the size of his palm and numerous smaller red spots. The skin in these areas was dry and tanned. He was treated with antibacterial salves on an outpatient basis and returned to work after 9 days, though some residual redness remained.

In the other two cases reported, the patients received two separate exposures to epichlorohydrin. In one case, a 32 year old male chemical worker spilled epichlorohydrin into his right safety shoe. The shoe was immediately removed and the foot rinsed with warm water; however, a spotty redness appeared over the ball and base joint of the large toe and he was admitted to the hospital within 2 hours of exposure. He was treated with saline solution compresses and discharged after 5 days. Thirteen days after the first exposure, he spilled epichlorohydrin in his left shoe. There was a slight burning sensation in the foot during the night, but he returned to work the following day. A blister developed 3 days following exposure. Upon admission to the hospital, he was found to have a 2x1 cm blister filled with yellow fluid, accompanied by reddening and swelling of the foot. Local treatment with saline solution and ointments was effective and the patient was dismissed from the hospital after 2 weeks.

The last case involved a 19 year old male chemical worker who accidentally poured epichlorohydrin on his left shoe. Red spots appeared on the dorsum of the foot within 6 hours, followed by the development of burning, itching, blisters, and skin erosion. This condition was initially treated on an outpatient basis by opening the blisters and applying a topical anesthetic and a corticoid salve, but the redness continued to intensify and the worker was admitted to the hospital 10 days following exposure. At the time of admission, a severe, 5 cm diameter skin erosion was observed on the dorsum of the foot, the temperature was slightly elevated and the lymph nodes in the left groin were painful and enlarged. Staphylococcus aureus was cultured from the skin erosion and the antistreptolysin titer was negative. This infection responded to penicillin injections and the skin erosion was treated with compresses and corticoid salves. Two years following the first exposure, this patient worked with epichlorohydrin for 3 days. During this time, he wore protective rubber gloves, onto which he spilled epichlorohydrin. After 3 days of work, the patient noticed burning, swelling, reddening and blister formation on the fingers of both hands. He was admitted to the hospital and treated with bland salves and metal foil bandages. The lesions responded to this treatment; however, redness was still apparent 18 days after exposure.

Inhalation Exposure. Compared to the cases of dermal exposure to epichlorohydrin, more pronounced toxicity was evident in a case with inhalation exposure. Schultz (1964) described the case of a 39 year old worker who was exposed to epichlorohydrin gas when the closure on a tank in the area in which he was working failed. He was momentarily paralyzed, then fled the area. He suffered an initial burning of the eyes and throat which intensified over the following hour. Swelling of the face, nausea, vomiting and severe headache developed during the remainder of the day, followed by shortness of breath during the night. He was admitted to the hospital with an enlarged liver and irritation of the mucosal lining of the upper respiratory tract. Two days following exposure, the liver was significantly enlarged and jaundice developed. The serum bilirubin (3.44 mg/100 mℓ) was almost [illegible] times the upper limit of the normal range and urobilinogen was detected in the urine. The jaundice gradually subsided and the patient was released from the hospital after 18 days. Bronchitis and noticeable liver dysfunction, accompanied by abnormal urinary levels of urobilin and urobilinogen were found at the 5 and 8 month follow-up examinations. Two years after the exposure, the patient was found to have delayed sulfobromophthalein elimination, increased galactase excretion and elevated urinary urobilin, bilirubin and urobilinogen. A liver biopsy revealed diffuse, severe, fatty degeneration. The patient also had chronic asthmatiform bronchitis which was considered to be the result of the epichlorohydrin exposure and elevated blood pressure which was not considered to result from the exposure to epichlorohydrin.

Epidemiological Studies

Several investigators have studied the frequency of chromosomal abnormalities in peripheral lymphocytes from workers occupationally exposed to epichlorohydrin (see Chapter V; Section on In Vitro). Workers exposed to epichlorohydrin had an increased incidence of chromosomal aberrations, mostly in the form of chromatid and chromosomal breaks. Other effects on blood cells have been reported by Sram et al. (1981) who examined blood samples from a group of 28 workers who had been occupationally exposed to epichlorohydrin for 4 years (Sram et al., 1980). The most common effect was a decreased hemoglobin concentration (10.8-13.2 g %/100 mℓ), which occurred in 16/28 workers. Decreased erythrocyte counts (3.7-4.1x$10^{12}/\ell$) and decreased leukocyte counts (3.4-4.4x$10^{9}/\ell$) each occurred in 5 workers.

The effects of epichlorohydrin on testicular function and fertility in experimental animals (Chapter V; Section on Teratogenicity) has led to concern about the effects of occupational exposures on human reproductive performance. Venable et al. (1980) investigated the fertility status of male employees at Dow Chemical Company's Texas Division who were involved in the production of chlorinated three-carbon compounds. Seventy-nine out of 123 employees volunteered for the study. Fifteen of these were subsequently excluded for medical reasons, such as a prior history of mumps orchitis, vasectomy, radiation treatment, etc. The control group consisted of 74 volunteers who had not worked in the production of industrial chemicals for at least 5 years, 11 of whom were excluded for medical reasons. The duration and extent of exposures were estimated from industrial hygiene records. The major variables studied were sperm count and percent normal sperm forms. The results of this study did not indicate any detrimental effect on fertility when comparisons were made between the control and exposed groups, or within the exposed group between different exposure levels, different durations of exposure, or both.

Milby et al. (1981) investigated testicular function among 44 epichlorohydrin workers at the Shell Chemical Corporation's Deer Park Chemical Plant at Deer Park, Texas and 84 epichlorohydrin workers at the Norco Chemical Plant at Norco, Louisiana. The control group consisted of 90 chemical plant workers who had been included in previous studies and who had not been exposed to agents known to be harmful to the testes. Three semen samples were requested from each subject, each obtained at home following 48 hours of sexual continence. No significant differences were found between control and exposed populations in sperm count, testosterone levels, FSH levels, or LH levels. There were no detectable differences in any of the parameters measured that were related to duration and/or extent of exposure. The authors concluded that "this study provided no evidence that exposure to ECH at the levels existing at the plants included is related to sperm count suppression."

Enterline (1978, 1980, 1981, 1982) discusses the results of a retrospective mortality study of workers exposed to epichlorohydrin, submitted to the Shell Oil Company in 1978 and presents a reanalysis of the data, taking into account sequential exposure to other industrial chemicals. The study group consisted of 864 male workers believed to have been exposed to epichlorohydrin for "more than one quarter during the years 1948-1965." The SMR for all causes of death was low, but there appeared to be a slight excess in deaths due to lung cancer, as compared to the general population and this was included in the 1978 report. Reanalysis of the data indicated that the excess in lung cancer deaths was clustered in a group of workers who were involved in the production of isopropyl alcohol prior to being involved in the production of epichlorohydrin. The data set in this study was too limited to clearly define the relationship between exposure to isopropyl alcohol and/or epichlorohydrin and the subsequent development of lung cancer. This data did, however, suggest that sequential exposures to these compounds constituted a greater risk than did exposure to either one individually.

The effect of epichlorohydrin exposure on mortality has also been investigated by the Dow Chemical Company (Dow, 1979). The study group consisted of 553 white male full-time employees of the Dow Chemical Company who were potentially exposed to epichlorohydrin for at least 1 month between October, 1957 and November, 1976. This group was further subdivided into four smaller categories: those with exposure to >1 ppm of both epichlorohydrin and allyl chloride, those with exposure to >1 ppm epichlorohydrin but not exposure to allyl chloride, those whose exposure was <1 ppm for both epichlorohydrin and allyl chloride, and those who were exposed to no more than 1 ppm epichlorohydrin with no exposure to allyl chloride. Exposures were calculated as an 8-hour TWA. A total of 12 deaths occurred in the study cohort, 2 due to cancer, 5 due to cardiovascular disease and 5 due to accidents. With the exception of one individual who died due to accidental trauma, all of the deceased had a positive history of smoking. Exposure among these 12 individuals ranged from 1 month to 17 years, 4 months, with a mean of 3 years, 3 months.

This study did not implicate epichlorohydrin as a human carcinogen. In contrast to the Enterline (1982) study, no deaths were attributed to leukemia or respiratory cancer. Due to the small numbers of deaths in this study, it is impossible to draw any firm conclusions as to the relationship between exposure to epichlorohydrin and mortality.

High Risk Subpopulations

Because of the effects of epichlorohydrin on testicular function in rats, adult males might be considered to be a high risk subpopulation. The effective doses in rats, however, were higher compared to approximate occupational exposure levels, and there were no-effect levels for male reproduction in the rat studies. Furthermore, epidemiologic studies have not shown an association between exposure to epichlorohydrin and reproductive performance in men. Hence, there seems to be insufficient evidence for considering men as high-risk population for reproductive effects at occupational or environmental levels of epichlorohydrin.

Summary

Acute toxic responses have been reported following both dermal (Ippen and Mathies, 1970) and inhalation (Schultz, 1964) exposures. Dermal exposure is characterized by an initial redness and itching or burning sensation. With time the redness intensifies and the tissue becomes swollen and blistered. Recovery is slow, sometimes leaving the skin with a rough texture. Inhalation exposure also produces local irritation, but appears to be more likely to produce systemic effects than is dermal exposure. The initial symptoms observed were burning of the eyes and throat, swelling of the face, nausea, vomiting and severe headache. Long-term effects were due primarily to damage to the liver and kidney. These effects were still present 2 years after the exposure in the case report by Schultz (1964).

Chronic exposure to epichlorohydrin has been implicated in a number of effects. Increased incidences of chromatid and chromosomal breaks are often observed in peripherial lymphocytes of epichlorohydrin workers. Other effects on blood cells include decreased hemoglobin concentration, decreased erythrocyte count and decreased leukocyte counts (Sram et al., 1980).

Venable et al. (1980) and Milby et al. (1981) have investigated the effects of occupational exposure to epichlorohydrin of fertility and testicular function. They found no evidence that epichlorohydrin reduced testicular function or fertility at the exposure levels commonly occurring in industry.

The effects of occupational epichlorohydrin exposure on mortality have been studied by the Shell Oil Company (Enterline, 1982) and by the Dow Chemical Company (Dow, 1979). The Shell study reported a slight, but not statistically significant, increase in the incidence of deaths due to lung cancer. These deaths were clustered in a group that had been sequentially exposed to isopropyl alcohol and epichlorohydrin. There is no convincing evidence that occupational exposure to epichlorohydrin results in an increased mortality.

VII. MECHANISMS OF TOXICITY

The mechanisms of toxicity of epichlorohydrin have been the subject of a number of studies, particularly as related to the antifertility effects noted in male animals. Since the compound and the least two of its metabolites are alkylating agents, its reactions with DNA have also been investigated. This chapter reviews the available data from these studies.

Alkylation of Cellular Macromolecules

Epichlorohydrin is a bifunctional alkylating agent, with electophilic carbons at positions 1 and 3. In addition, the major metabolite of epichlorohydrin, α-chlorohydrin, is slightly electrophilic, and the other metabolite, glycidol, is also an electrophilic epoxide (see Metabolism Section in Chapter III). There are, thus, numerous ways in which epichlorohydrin and its metabolites may interact with cellular nucleophiles.

Hemminki (1979) and Hemminki et al. (1980) investigated the covalent interactions of epichlorohydrin with DNA. Hemminki (1979) used a fluorescence assay to measure the alkylation of guanine by a number of epoxides, including epichlorohydrin. He found that the rates of reaction were in the order of deoxyguanosine>single-stranded DNA>double-stranded DNA. He also found that, among the epoxides tested, mutagenic potency was directly proportional to reactivity. Analysis of the products of the reaction of epichlorohydrin with DNA (Hemminki et al., 1980) revealed three adducts. The major adduct was either 1,7- or 1,9-diaklylguanine. The two minor adducts were identified as 7-aklylguanine and N-6-alkyladenosine. Glycidol was also studied and was found to produce the same adducts as epichlorohydrin, plus an N-3-alkylcytidine adduct. These studies indicate that the alkylating properties of epichlorohydrin and its metabolites may be involved in the mutagenicity and carcinogenicity of epichlorohydrin.

It has also been suggested that the antifertility effects of epichlorohydrin are due to its alkylating potential (Jones et al., 1969; Kalla and Bansal, 1977). Jones et al. (1969) originally proposed that the antifertility effects of epichlorohydrin could be the result of binding to cysteine in the sperm. Kalla and Bansal (1977) demonstrated the inhibition of both glycolysis and motility due to alkylation of spermatozoan cysteine.

Metabolic Formation of γ-Chlorohydrin-1-Phosphate

The metabolic formation of α-chlorohydrin-1-phosphate has also been suggested as a possible mechanism in the antifertility effects of epichlorohydrin. Mohri et al. (1975) found that the glycolytic pathway is particularly sensitive to inhibition by γ-chlorohydrin in spermatozoa. In subsequent investigations, they determined the concentrations of various glycolytic intermediates following incubation of ram spermatozoa with 15 mM fructose in the presence or absence of 3 mM γ-chlorohydrin. The inhibition of glycolysis was found to be due to the inhibition of glyceraldehyde-3-phosphate dehydrogenase; however, γ-chlorohydrin did not inhibit the purified enzyme, implying that a metabolite of γ-chlorohydrin was responsible for the inhibitory effect. They found that phosphorylation of γ-chlorohydrin at the C1 position resulted in a compound which inhibited glyceraldehyde-3-phosphate dehydrogenase directly, and suggested that this was the likely active metabolite. The inhibition of this enzyme was further investigated by Mashford and Jones (1978). They found that γ-chlorohydrin-1-phosphate, but not γ-chlorohydrin, epichlorohydrin, or glycidol, inhibited glyceraldehyde-3-phosphate dehydrogenase in a competitive manner, thus supporting the view that γ-chlorohydrin-1-phosphate is the active metabolite responsible for the antifertility effects of epichlorohydrin.

Fitzpatrick et al. (1978) studied the inhibitory effects of S(+)- and R(-)-α-chlorohydrin, as well as the racemic mixture, on glyceraldehyde-3-phosphate dehydrogenase. They found that S(+)-α-chlorohydrin was almost four times as potent as the racemic mixture, while the R(-) isomer was not inhibitory. They concluded that the actual inhibitory metabolite was S(+)-α-chlorohydrin-1-phosphate.

Metabolic Formation of Oxalic Acid

One of the products of epichlorohydrin metabolism is oxalic acid (see Metabolism Section in Chapter III). Jones et al. (1978) suggest that oxalic acid, in the form of calcium oxalate, is responsible for the renal toxicity of epichlorohydrin and for the formation of spermatoceles observed following high doses of epichlorohydrin. They indicated that calcium oxalate caused a type of renal glomerular nephritis which was responsible for the diuretic action of both α-chlorohydrin and β-chlorolactate.

Interactive Effects

Interactive effects between epichlorohydrin and other compounds have not been clearly demonstrated. In an epidemiological study (Enterline, 1982), sequential exposure to isopropyl alcohol and epichlorohydrin was implicated in the induction of lung cancer, but the nature of the interaction, if any existed, could not be elucidated from the available data.

A number of investigators have suggested that conjugation with glutathione, catalyzed by glutathione-S-transferases, is a major detoxification pathway for epichlorohydrin (Fjellstedt et al., 1973; Hayakawa et al., 1975; Andersen et al., 1980). Exposure to chemicals which deplete cellular glutathione should, therefore, increase the toxicity of epichlorohydrin. Also, exposure to epichlorohydrin should deplete glutathione, thus increasing the toxicity of other compounds, such as acetomenephen, which are detoxified by conjugation with glutathione.

Summary

Three mechanisms have been proposed by which epichlorohydrin may exert its toxicity. Epichlorohydrin and at least two of its metabolites are alkylating agents. Thus, these compounds are able to bind covalently to many cellular nucleophiles. Alkylation of cellular DNA has long been recognized as a mechanism for the induction of mutations and for the induction of tumors. Jones et al. (1969) and Kalla and Bansal (1977) have suggested that alkylation of cysteine moieties in spermatozoa may be partially responsible for the antifertility effects of epichlorohydrin.

The metabolic formation of α-chlorohydrin-1-phosphate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, has also been implicated in the antifertility effects of epichlorohydrin (Mohri et al., 1975; Mashford and Jones, 1978). The absolute spatial distribution of the molecule was found to be important to the inhibitory property of epichlorohydrin, with the S(+) isomer being the active form (Fitzpatrick et al., 1978). The metabolite responsible for the inhibition of glygeraldehyde-3-phosphate dehydrogenase is, therefore, S(+)-α-chlorohydrin-1-phosphate.

Another metabolite of epichlorohydrin is oxalic acid which, as the calcium salt, is believed to be responsible for the renal toxicity of epichlorohydrin (Jones et al., 1978). They also suggested that calcium oxalate was involved in the formation of spermatoceles following large doses of epichlorohydrin.

There are no well documented interactive effects between epichlorohydrin and other compounds; however, two possible interactions have been suggested. Based on epidemiological evidence, Enterline (1982) has suggested that sequential exposure to isopropyl alcohol and epichlorohydrin may result in a greater risk of lung cancer than does exposure to either compound alone. Epichlorohydrin may interact with other compounds through the formation of glutathione conjugates. Since this is a detoxification reaction for epichlorohydrin (Andersen et al., 1980), exposure to compounds which deplete glutathione could increase the toxicity of epichlorohydrin. At the same time, exposure to epichlorohydrin may deplete glutathione, thus increasing the toxicity of other compounds which are detoxified by conjugation with glutathione.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an acceptable daily intake (ADI) is calculated. An adjusted acceptable daily intake (AADI) and health advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg/}\ell$$

where: NOAEL = no-observed-adverse-effect level

LOAEL = lowest-observed-adverse-effect level

Body weight = 70 kg for adult or 10 kg for child

Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children

Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identifing a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (epichlorohydrin is a carcinogen), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for non-continuous animal feeding, non-lifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

The NAS (1980) SNARLs for 1- or 7-day exposures to epichlorohydrin are 0.84 and 0.53 mg/ℓ, respectively. An ADI or a cancer risk was not calculated by the NAS (1980). The ACGIH has recommended a TLV of 2 ppm (10 mg/m^3) and a STEL of 5 ppm (20 mg/m^3) (ACGIH, 1980, 1982). Current OSHA standards allow a TWA occupational exposure of 19 mg/m^3 (29 CFR 1910.1000); however, they are currently considering lowering this value to 0.5 ppm (2 mg/m^3) with a ceiling value of 15 ppm (60 mg/m^3) for 15 minutes. Occupational standards in other countries range from 0.26 ppm in Russia and Czechoslovakia to 3.6 ppm in the Federal Republic of Germany (Sram et al., 1980). Epichlorohydrin has not been regulated under the Safe

Drinking Water Act; however, discharge of >1000 pounds (454 kg) into navigable waters is prohibited under the Clean Water Act (40 CFR 116). Epichlorohydrin is also classified as a "hazardous waste" by the U.S. EPA and quantities exceeding 100 kg must be disposed of in a special landfill (40 CFR 261; 40 CFR 122).

Noncarcinogenic Effects

As described in the Chapter V general toxicity section (Table V-1), epichlorohydrin is toxic in a wide variety of species (rats, mice, guinea pigs, rabbits, cats and dogs) and following many types of exposure (oral, inhalation, intraperitoneal, subcutaneous, dermal, corneal and intravenous). In those cases where direct comparisons can be made, epichlorohydrin appears to be approximately equitoxic between species. There is not, therefore, any one species which is obviously preferable for use in deriving estimates of risk.

1-Day HA. The number of studies in which the acute toxicity of low doses of epichlorohydrin has been determined, and which are, therefore, useful for quantitative risk assessment, are limited.

Van Esch (1981) exposed groups of five male and five female Wistar rats to epichlorohydrin by gavage at levels of 10, 20, 40 and 80 mg/kg bw, 5 days/week for 2 weeks. In males, decreased erythrocyte count and increased kidney/body weights were observed at all dose levels, while degenerative changes in the kidneys were reported for both males and females at doses ≥40 mg/kg. Kidney/body weights in females were increased with the three highest dose levels. In addition, Van Esch (1981) reported on two reproduction studies using similar administration schedules. In the first study, groups of four female and two male rats were exposed to epichlorohydrin at doses of 5, 10 or 20 mg/kg bw for 3 months. The exposure started 4 days prior to mating, and fertility was adversely affected (no pregnancies) only in the high-dose group. In the second study, the rats in groups of 20-30 females and 10-15 males were administered epichlorohydrin at doses of 0, 2 or 10 mg/kg bw and allowed to produce two generations. In this study, exposure began 10 days prior to mating. At the 10 mg/kg level, the fertility index was reduced to 3.3% as compared to 55% in the controls. By cross-mating treated (10 mg/kg group) and control animals, it was determined that epichlorohydrin exposure had adverse effects on male reproductive capacity. This suggests that at least part of the reduced fertility index might have resulted from the male exposure. With respect to the effects of the exposure on reproductive capacity, 2 mg/kg was a NOEL.

In other studies, epichlorohydrin has been found to produce infertility in males. Hahn (1970) administered oral doses of 15 mg epichlorohydrin/kg bw/day to male Sprague-Dawley rats for 12 consecutive days. At various times during and after the treatment period, two pre-esterous females were placed in the cage with each male. Within 1 week, epichlorohydrin treatment resulted in infertility in the male rats. This effect was reversible within 1 week after treatment was discontinued. This effect was confirmed by Cooper et al. (1974) using male Wistar rats and slightly higher doses of epichlorohydrin (20 mg/kg bw/day for 5 consecutive days). At higher doses (50 mg/kg bw/day) the infertility produced was not reversed within 10 weeks after treatment was discontinued.

Quast et al. (1979b) exposed groups of five male and five female Sprague-Dawley rats, Fischer 344 rats and B6C3F$_1$ mice to atmospheres containing 100 ppm (378 mg/m^3) epichlorohydrin, 6 hours/day, 5 days/week, for a total of 9 exposures for 12 days. By assuming breathing volumes of 0.0093 m^3/hour (0.223 m^3/day) for rats and 0.0016 m^3/hour (0.039 m^3/day) for mice, body weights of 0.35 kg for rats and 0.03 kg for mice, and an absorption efficiency of 0.5, and by converting to an equivalent exposure of 7 days/week rather than 5 days/week, this concentration corresponds to a dose of 21.5 mg/kg bw/day for rats and 43.9 mg/kg bw/day for mice (see Quantification of Noncarcinogenic Effects Section). No evidence of eye or nasal irritation was detected during the exposure periods; however, a slight nasal discharge was noted in the rats on removal from the exposure chamber. Signs of respiratory distress, reduced fecal excretion and an apparent decreased food consumption were observed during the first 5 days and the last 4 days of exposure, with partial recovery over the weekend. A significant decrease in body weight was observed in both rats and mice. Histological examination revealed degeneration, inflammation, hyperplasia and squamous metaplasia of the nasal turbinates in both rats and mice. Both strains of rats, but not the mice, developed degenerative changes in the kidneys.

Fomin (1966) estimated the olfactory threshold of epichlorohydrin and the effect of epichlorohydrin on light sensitivity and cerebral electrical activity in 18 subjects between 17 and 33 years of age. The length of exposure was not reported, but, based on Figure 1 in the paper, the maximum exposure time may have been 15-20 minutes. Assuming an exposure of 20 minutes, an average weight of 70 kg, a breathing volume of 0.83 m^3/hour (20 m^3/day), and an absorption efficiency of 0.5, concentrations of 0.2

and 0.3 mg/m³ correspond to doses of 0.0004 and 0.0006 mg/kg bw (see Quantification of Noncarcinogenic Effects Section). He reported an odor threshold of 0.3 mg/m³ for the most sensitive subjects and a subthreshold level of 0.2 mg/m³. No effects on light sensitivity were noted at any concentration tested. An epichlorohydrin concentration of 0.3 mg/m³ produced significant changes in cerebral electrical activity. No effects were observed following exposure to 0.2 mg/m³. Neither observation appears to have a clear toxicological significance and, therefore, was not considered in the derivation of a 1-day HA.

10-Day HA. The only study using the oral route of administration for periods between 30 and 90 days is the reproductive toxicity study in rats (Van Esch, 1981) described for the 1-day HA. Rats were observed to have decreased reproductive performance after 10 days of exposure to epichlorohydrin at a dose of 10 mg/kg bw, while at 2 mg/kg bw, no effects were observed. With continued exposure of both males and females for 3 months (5 days/week), there were no additional observations reported. For this 3-month study, 2 mg/kg was a NOEL.

A number of inhalation studies have been conducted for periods between 30 and 90 days. Laskin et al. (1980) observed inflammatory changes in the respiratory tract, and kidney lesions in a group of 140 male rats exposed to 100 ppm of epichlorohydrin 6 hours/day for 30 days. At lower exposures of 5, 25 and 50 ppm 6 hours/day for 70-90 days, Quast et al. (1979a) and John et al. (1983) reported damage to the respiratory tract in mice and rabbits exposed to $\geq$25 ppm epichlorohydrin. In rabbits, reproductive performance was asessed to be normal at all exposure levels. For rats, mice and rabbits, the 5 ppm exposure level represented a NOEL.

Fomin (1966, 1968) exposed groups of 15 male rats to atmospheres containing 0.2, 2.0 or 20 mg epichlorohydrin/m³ continuously for 98 days. At the highest concentration, epichlorohydrin produced a significant increase in the latent reaction time, as measured by the motor-defensive reaction in response to stimulation with an electric current, after 1.5 months of exposure. In addition, the fluorescence characteristics of the leucocytes, using acridine orange as the fluorochrome, were altered during the early portion of the study, but returned to normal by the end of the study. Blood nucleic acids were decreased and urinary coproporphyrin excretion was increased. Histological examination revealed emphysema, fine-focus pneumonia, small areas of edema and loosening and swelling of the adventitia of the blood vessels in the lungs. In the kidneys, extensive damage, described as protein degeneration of the epithelium of the convoluted tubules, was found. The veins of the heart were congested and intermuscular fine-focus hemorrhages were noted. Serious, but undescribed, damage to the neurons of the myelencephalon, cerebellum and ammon's horn of the brain was reported. At the intermediate concentration (2.0 mg/m³), the only effects noted were a slight temporary alteration in leucocyte fluorescence characteristics and a mild decrease in blood nucleic acids. No histological changes were noted. No effects were observed at an atmospheric concentration of 0.2 mg/m³.

Lifetime AADI. Several studies in which epichlorohydrin was administered for the lifetime of the animals were found in the available literature (Laskin et al., 1980; Kawabata, 1981; Konishi et al., 1980). Non-peer reviewed results are available for another study (Van Esch, 1981; 1982).

Groups of 100 male Sprague-Dawley rats were exposed to 10 ppm (38 mg/m^3) or 30 ppm (114 mg/m^3) epichlorohydrin, 6 hours/day, 5 days/week, for the lifetime of the animals (136 weeks; Laskin et al., 1980). Assuming an absorption efficiency of 0.5, a breathing volume of 0.0093 m^3/hour and an average weight of 0.35 kg, these exposures correspond to oral doses of 2.16 and 6.49 mg/kg bw/day (see Quantification of Noncarcinogenic Effects Section).

In the high-dose group, significant weight loss was observed after 40 weeks of exposure. Pulmonary congestion, bronchiolectasis and pneumonia were observed in both treatment groups. The incidences of renal damage were 65 and 37% for the groups exposed to 30 and 10 ppm, respectively, compared with a 14% incidence in untreated controls and a 24% incidence in sham-treated animals. The severity of renal damage, described as mainly tubular degeneration changes, was greater in treated animals than in controls. Therefore, a LOAEL of 10 ppm (2.16 mg/kg bw/day) is evident in this study.

In the study reported by Konishi et al. (1980) and Kawabata (1981), epichlorohydrin was administered to groups of 18 male Wistar rats in the drinking water as described in the Carcinogenicity Section in Chapter V. The total doses were reported as 0, 5.0, 8.9 and 15.1 g during 81 weeks of treatment. Based on the weight data presented in the paper, the approximate average daily doses were 0, 18, 39 and 89 mg/kg bw/day. This was principally a carcinogenicity study with only limited toxicology data being presented. Weight gain was decreased in all treatment groups, as compared to the controls, kidney and pancreas/body weight ratios were increased, liver, testes and heart/body weight ratios were decreased and serum cholesterol and neutral lipid levels were elevated. In addition, Van Esch (1981) administered epichlorohydrin by gavage 5 days/week at doses of 0, 2 and 10 mg/kg bw to groups of 50 male and 50 female rats for 104 weeks. The only treatment-related effects reported were a dose-related decrease in white blood cell counts in females, and neoplastic and hyperplastic lesions in the stomach of all treatment groups. This was a preliminary report, and the histologic evaluation of other tissues and organs had not yet been completed.

Quantification of Noncarcinogenic Effects

1-Day HA. The reproductive toxicity study by Van Esch (1981) can be used to derive the 1-day HA. In this study, male and female rats were given epichlorohydrin by gavage 5 days/week at doses of 0, 2 or 10 mg/kg bw. Exposure was started 10 days prior to mating and continued until the F_{1b} generation was produced. The fertility index at the first mating was reduced in the high-dose group but not in the low-dose group. The study of Hahn (1970) which reported infertility in male rats exposed by gavage to epichlorohydrin at 15 mg/kg/day for 12 days supports an assumption that at least a portion of the reduced fertility index observed by Van Esch (1981) was the result of infertility in the males associated with the 10-day exposure prior to mating. In this study, 2 mg/kg bw was a NOEL for reproductive effects and is appropriate for use in deriving the 1-day HA.

Using the NOEL of 2 mg/kg and a UF of 100 for extrapolating from an animal study to humans, the 1-day HAs for an adult and child can be calculated as follows:

For an adult:

$$\text{HA} = (2\ \text{mg/kg/day} \times 70\ \text{kg} \times 5/7\ \text{days}) \div (100 \times 2\ \ell\text{/day}) = 0.5\ \text{mg/}\ell$$

For a child:

$$\text{HA} = (2\ \text{mg/kg/day} \times 10\ \text{kg} \times 5/7\ \text{days}) \div (100 \times 1\ \ell\text{/day}) = 0.14\ \text{mg/}\ell$$

where:

2 mg/kg = a NOEL for reproductive toxicity
70 kg = the assumed weight of an adult
10 kg = the assumed weight of a child
100 = a UF appropriate for a NOEL from an animal study
2 ℓ/day = the amount of water consumed/day by an adult
1 ℓ/day = the amount of water consumed/day by a child
5/7 days = expanding dose from 5 to 7 days

Although the antifertility effect in male rats in the Van Esch (1981) study relates to men as a specific sensitive subpopulation for this effect, this study is preferred for calculations of a 1-day HA for the general population because of its design with oral short-term exposure and its demonstration of no-effect and effect levels. Additionally, the 2 mg/kg NOEL in the Van Esch (1981) study appears consistent with the dose responses in the overall Van Esch (1981) work where both systemic and reproductive effects were found with 10-day oral exposures to 10 mg/kg of epichlorohydrin. Van Esch (1981) found NOELs of 5 and 10 mg/kg for infertility in male rats given epichlorohydrin by gavage for 4 days prior to mating; however, only 2 males and 4 females per group were used in this particular study whereas the Van Esch (1981) reproduction study with a 10-day treatment included 20 females and 10 males per group. The study used to calculate the 7-day SNARL by NAS (1980) was the study by Hahn (1970) where male rats were sterilized within 1 week by daily oral exposure to 15 mg/kg bw of epichlorohydrin. With a 1000-fold safety factor appropriate for this LOAEL, the calculation is:

$$(15 \text{ mg/kg/day} \times 70 \text{ kg}) \div (1000 \times 2 \text{ ℓ/day}) = 0.53 \text{ mg/ℓ}$$

In comparison, this number is almost equal to the HA obtained using the Van Esch (1981) study results.

10-Day HA. The study preferred as the basis for a 10-day HA is that of Van Esch (1981) in which a NOEL of 2.0 mg/kg bw (5 days/week) was reported for reproductive toxicity. Using the NOEL of 2 mg/kg, expanding this dose from 5 to 7 days to represent continuous exposure, and applying a UF of 100 for extrapolating an animal study to humans and for protection of the most sensitive members of the population, the 10-day HAs for an adult and a child can be calculated as follows:

For an adult:

$$\text{HA} = (2 \text{ mg/kg/day} \times 5/7 \text{ days} \times 70 \text{ kg}) \div (100 \times 2 \text{ ℓ/day}) = 0.5 \text{ mg/ℓ}$$

For a child:

$$\text{HA} = (2 \text{ mg/kg/day} \times 5/7 \text{ day} \times 10 \text{ kg}) \div (100 \times 1 \text{ ℓ/day}) = 0.14 \text{ mg/ℓ}$$

Applying the 19 mg/m³ no-effect level in the John et al. (1983) and Quast et al. (1979a) studies, and the assumptions for converting inhalation exposure to oral exposure in the document, the calculations for an animal dose would be:

$$d_a = (19 \text{ mg/m}^3 \times 0.0093 \text{ m}^3\text{/hr} \times 6 \text{ hr/day} \times 5/7 \times 0.5) \div 0.35 \text{ kg} = 1.08 \text{ mg/kg/day}$$

where:

d_a = animal dose
0.0093 m^3 = amount of air breathed by a rat/hour
6 hr/day = a 6-hour exposure each day
5/7 = adjust from a 5 days/week exposure to 7 days/week
0.5 = the assumed inhalation absorption factor
0.35 kg = the assumed weight of a rat

From this dose of 1.08 mg/kg/day, and the use of a 100-fold UF, a 10-day HA would be:

For an adult:

$$HA = (1.08 \text{ mg/kg/day} \times 70 \text{ kg}) \div (100 \times 2 \text{ } \ell\text{/day}) = 0.38 \text{ mg/}\ell$$

For a child:

$$HA = (1.08 \text{ mg/kg/day} \times 10 \text{ kg}) \div (100 \times 1 \text{ } \ell\text{/day}) = 0.11 \text{ mg/}\ell$$

Although less desirable because of uncertainty in converting inhalation-dose effect to oral-dose effect, these numbers approximate the numbers calculated with the Van Esch (1981) data. A no-effect level of 19 mg/m^3 in rabbits, which would yield a smaller oral dose, was observed by John et al. (1983); but the effect of lesions in the nasal turbinates is an unlikely consequence from oral exposure.

<ins>Lifetime AADI</ins>. Of the reviewed studies in which the effects of long-term exposure to epichlorohydrin were investigated (Laskin et al., 1980; Konishi et al., 1980; Kawabata, 1981), the Laskin et al. (1980) study was selected because the design included lifetime exposures and pathological

examination of all animals. Forestomach hyperplasia, at an incidence of 77.8-100% in all three treatment groups, and papillomas and carcinomas of the forestomach in the two highest dose groups were found in the study by Kawabata (1981) and Konishi et al. (1980), and since the hyperplasia could be considered a preneoplastic effect, it would be questionable to use this effect in the low-dose group for calculating a noncarcinogenic HA for drinking water exposure. The LOAEL based on renal damage of 2.16 mg/kg bw/day estimated from the data in the Laskin et al. (1980) study was, therefore, used to derive a lifetime AADI. Using a UF of 1000 for an animal LOAEL and assuming a daily water consumption of 2 ℓ for a 70 kg adult, a lifetime AADI may be calculated:

$$\text{AADI} = (2.16 \text{ mg/kg bw/day} \times 70 \text{ kg}) \div (1000 \times 2 \text{ ℓ/day}) = 0.076 \text{ mg/ℓ}$$

This AADI is equivalent to an ADI of ~0.15 mg/day or ~0.0022 mg/kg bw/day.

Carcinogenic Effects

Based on an analysis of the available literature, the animal evidence is sufficient to conclude that epichlorohydrin is carcinogenic in animals and probably carcinogenic in humans (IARC, 1982). The study that is most pertinent for estimating carcinogenic potential from drinking water exposure is that reported by Konishi et al. (1980) and Kawabata (1981). Groups of 18 male Wistar rats were given drinking water containing 0, 375, 750 or 1500 ppm (0, 375, 750 or 1500 mg/ℓ) epichlorohydrin for 81 weeks. At various times during this period, exposure was interrupted due to toxic effects (see the Carcinogenicity Section in Chapter V). Animals that died during treatment were excluded from the final analysis. In the animals that survived the treatment period, the most common findings at the terminal sacrifice were tumors of the forestomach. These results are summarized in Table VIII-1. The tumors were described, macroscopically, as "uneven, irregular, protuberant large tumors and countless small, nodular tumors having a grayish-white color." These tumors were identified histologically as hyperplasia involving the horny layer, proliferation of the squamous epithelium cells and sometimes multiple stratification of the basal cells, papillomas consisting of squamous epithelial tissue projecting into the lumen, and squamous cell carcinomas consisting of highly differentiated tumor cells, accompanied by keratinization, which had infiltrated the basal membrane.

The U.S. EPA's Carcinogen Assessment Group (CAG) has estimated a 95% upper-limit of risk (U.S. EPA, 1983) from an epidemiological study of workers at the Shell Oil Company (Enterline, 1982), an oral carcinogenicity study (Konishi et al., 1980; Kawabata, 1981) and an inhalation study (Laskin et al., 1980).

The 95% upper-limit unit risk estimates from the animal data are based on the linearized multistage nonthreshold extrapolation model. Justification for its use has been given previously (Federal Register, 1980). Basically, the use of the model tends to yield a higher estimate of risk, and is more conservative than alternate models such as the Probit or Weibull models. For this particular chemical and medium, however, it gives lower estimates than the one-hit model. CAG considers these risk estimates to be upper-limit estimates in the sense that the true risk is unlikely to be higher than the estimate, but may be considerably lower. The 95% upper-limit unit risk estimate (based on the human data) is derived by assuming that lung cancer in the cohort follows an exponential distribution and then calculating the confidence limit from the data.

TABLE VIII-1

Number of Forestomach Tumors and Tumor Incidence of Rats Given Epichlorohydrin*

Group Number	Dose ppm	No. of Rats		No. of Tumors in Forestomach/Rat				Histological Findings on Squamous Epithelium (%)		
					Tumor size (mm)					
		Initial	Effective	Total	2>	2≤>5	5≤	Hyperplasia	Papilloma	Carcinoma
1	0	18	10	0	0	0	0	0 (0)	0 (0)	0 (0)
2	375	18	9	5.6±8.4	5.4±8.2	0.1±0.3	0	7 (77.8)	0 (0)	0 (0)
3	750	18	10	9.9±12.8	8.3±12.0	1.2±1.2	0.4±0.5	9 (90.0)	1 (10.0)	1 (10.0)
4	1500	18	12	32.8±24.0	27.6±21.3	4.4±3.6	0.8±1.0	12 (100.0)	7 (58.5)	2 (16.7)

*Source: Kawabata, 1981

In analyzing the epidemiologic study of Shell epichlorohydrin workers, because of the fact that the observed increase in lung cancer deaths was not statistically significant and the confounding effect of exposures to other chemicals, only an upper-bound to the risk could be calculated. In addition, only very rough estimates of the exposure levels could be made. With these limitations in mind, CAG calculated that the 95% upper-limit of risk of death from lung cancer from breathing 1 $\mu g/m^3$ epichlorohydrin for 70 years is 3.9×10^{-5} $(\mu g/m^3)^{-1}$.

The carcinogenic risk from epichlorohydrin exposure in drinking water was calculated from the data of Konishi et al. (1980) and Kawabata (1981). In analyzing the data, the incidences of papillomas and carcinomas of the forestomach were combined to yield incidences of 0/10, 0/9, 2/10 and 9/12 for the control, 375, 750 and 1500 ppm dose groups. The incidence in the high-dose group was significantly greater than the controls ($p<0.001$). Based on these data, CAG derived a human q_1^* of 9.9×10^{-3} $(mg/kg/day)^{-1}$.

Similarly, a human q_1^* of 1.2×10^{-6} $(\mu g/m^3)^{-1}$ was calculated, based on the inhalation study of Laskin et al. (1980), using only the control and two lower exposure groups. At these levels the incidences of nasal squamous cell carcinoma (0/100 and 1/100 for exposure of 10 and 30 ppm, respectively) were not significantly increased in the lifetime exposures. When the high exposure response (15/140 for nasal squamous cell carcinomas plus two nasal papillomas) was included, the time-to-tumor response model yielded 95% upper-limit estimates ~10 times higher. These higher values are considered pertinent only for high level short-term exposures and are presented for informational purposes.

Quantification of Carcinogenic Effects

The study of Konishi et al. (1980) and Kawabata (1981) provides appropriate data for a quantitative risk assessment based on the relevant route of exposure and the observed dose-response. Using the calculated q_1^* of 9.9×10^{-3} $(mg/kg/day)^{-1}$, the 95% lower-limit lifetime dose associated with a 10^{-5} risk level may be calculated:

$$mg/day = 70\ kg \times 10^{-5}/q_1^*$$

where:

70 kg is the assumed weight of an average man

thus:

$$mg/day = 70 \times 10^{-5}/9.9 \times 10^{-3} = 7.07 \times 10^{-2}$$
$$= 70.7\ \mu g/day$$

Assuming an average water consumption of 2 ℓ/day, this risk level corresponds to a water concentration of 35.4 μg/ℓ. Corresponding levels for 10^{-6} and 10^{-4} are 3.54 and 354 μg/ℓ, respectively.

Special Considerations

Synergistic Effects. Epichlorohydrin is not known to produce synergistic effects with any other compound. In an epidemiological study, Enterline (1982) has indicated that sequential exposure to isopropyl alcohol and epichlorohydrin may increase lung cancer incidences relative to exposure to either compound alone, but the observed increases were not statistically significant. It may also be proposed that exposure to glutathione-depleting agents should increase the toxicity of epichlorohydrin, but this has not been demonstrated.

High Risk Subpopulations. The available data does not suggest the existence of any particular high risk subpopulation. It may be speculated that, due to the local irritation produced by epichlorohydrin, individuals with pre-existing conditions such as gastric ulcers may suffer increased discomfort due to oral exposure to epichlorohydrin, but no data were found to support this speculation.

Organoleptic Properties. A threshold for odor perception of epichlorohydrin of 0.5-1.0 mg/ℓ and a threshold for its irritant action of 0.1 mg/ℓ was cited by the NAS (1980).

Summary

The HAs derived for epichlorohydrin are summarized in Table VIII-2. The recommended 1-day and 10-day HAs incorporated a broad data base on epichlorohydrin toxicity from short-term exposures to develop HAs which are protective against potential acute effects from exposure to epichlorohydrin. The recommended lifetime AADI is derived from a chronic inhalation exposure study in rats where noncarcinogenic toxicity was observed with lifetime exposures. Since epichlorohydrin is a demonstrated carcinogen in animals, the 95% lower-limit drinking water criteria levels of 3.54 and 35.4 µg/ℓ of epichlorophydrin corresponding to lifetime cancer risks of $1x10^{-6}$ and $1x10^{-5}$, respectively, have been calculated under the assumptions of the multistage model. Thresholds of 0.5-1.0 mg/ℓ for odor perception and 0.1 mg/ℓ for irritation have bee reported for epichlorohydrin.

TABLE VIII-2

Health Advisories for Epichlorohydrin

Health Advisory	μg/ℓ
1-day (child)	140
1-day (adult)	500
10-day (child)	140
10-day (adult)	500
Lifetime AADI (toxicity)	76
Lifetime (10^{-6} excess cancer risk)	3.54
(10^{-5} excess cancer risk)	35.4

IX. REFERENCES

ACGIH (American Conferences of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values, 4th ed. (Includes Supplemental Documentations, 1981.) Cincinnati, OH.

ACGIH (American Conferences of Governmental Industrial Hygienists). 1982. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment. Cincinnati, OH.

Andersen, M., P. Kiel, H. Larsen and J. Maxild. 1978. Mutagenic action of aromatic epoxy resins. Nature. 276: 391-392.

Andersen, M.E., O.E. Thomas, M.L. Gargas, R.A. Jones and L.L. Jenkins, Jr. 1980. The significance of multiple detoxification pathways for reactive metabolites in the toxicity of 1,1-dichloroethylene. Toxicol. Appl. Pharmacol. 52(3): 422-432.

Bartsch, H., C. Malaveille, A.M. Camus, et al. 1980. Validation and comparative studies on 180 chemicals with S. typhimurium strains and V79 Chinese hamster cells in the presence of various metabolizing systems. Mutat. Res. 76: 1-50.

Bridges, B.A. 1978. On the detection of volatile liquid mutagens with bacteria: Experiments with dichlorvos and epichlorohydrin. Mutat. Res. 54: 367-371.

Bridie, A.L., C.J.M. Wolff and M. Winter. 1979. BOD and COD of some petrochemicals. Water Res. 13: 627-30.

Burns, L.H., D.M. Cline and R.R. Lassiter. 1981. Exposure Analysis Modeling System (EXAMS). Prepared by Environmental Research Laboratory, ORD, U.S. EPA, Athens, GA.

Carbone, P., G. Barbata, G. Margiotta, A. Tomasino and G. Granata. 1981. Low epichlorohydrin concentrations induce sister chromatid exchanges in human lymphocytes in vitro. Caryologia. 34(3): 261-266.

Carpenter, C.P., H.F. Smyth, Jr. and U.C. Pozzani. 1949. The assay of acute vapor toxicity and the grading and interpretation of results on 96 chemical compounds. J. Ind. Hyg. Toxicol. 31: 343-346.

Cooper, E.R., A.R. Jones and H. Jackson. 1974. Effects of alpha-chlorohydrin and related compounds on the reproductive organs and fertility of the male rat. J. Reprod. Fert. 39(2): 379-386.

Dabney, B.J., R.V. Johnston, J.F. Quast and C.N. Park. 1979. Epichlorohydrin -- Subchronic studies. III. Cytogenetic evaluation of bone marrow cells from rats exposed by inhalation to epichlorohydrin for four weeks. ICPEM (International Commission for Protection Against Environmental Mutagens and Carcinogens) Document No. 128. 15 p.

Dean, B.J. and G. Hodson-Walker. 1979. An in vitro chromosome assay using cultured rat-liver cells. Mutat. Res. 64: 329-337.

Dixit, V.P. 1977. Action of monochlorohydrin on epididymis of dog. Ind. J. Exp. Biol. 15: 233.

Doll, R. 1971. Weibull distribution of cancer. Implications for models of carcinogenesis. J. Roy. Stat. Soc. A13: 133-166.

Dow Chemical Company. 1979. Cytogenetic Findings from Epoxy Resins and Glycerine Employees. Technical Evaluation and Review Branch. Texas Division, Freeport, TX.

Eder, E., T. Neudecker, D. Lutz and D. Henschler. 1980. Mutagenic potential of allyl and allylic compounds. Structure activity relationship as determined by allkylating and direct in vitro mutagenic properties. Biochem. Pharmacol. 29: 993-998.

Enterline, P.E. 1978. Updated mortality in workers exposed to epichlorohydrin. Unpublished reports to Shell Oil Co., August 31, 1977; July 31, 1978, and letter to R.E. Joyner, Shell Oil Co., July 31. Letter from R.E. Joyner, corporate Medical Director, Shell Oil Co., to J.M. Lane, Acting Director, NIOSH, August 7.

Enterline, P.E. 1980. Cancer in men manufacturing isopropyl alcohol. Report attached to a letter from M.J. Slan, Shell Oil Co., to U.S. EPA, November 11. EPA Document Control No. OTS-1180-0069.

Enterline, P.E. 1981. Further update of mortality among workers exposed to epichlorohydrin. Report to Shell Oil Co., January 13. Submitted to U.S. EPA, Office of Toxic Substances. Docket No. 8EHQ-0878-0230 Suppl.

Enterline, P.E. 1982. Importance of sequential exposure in the production of epichlorohydrin and isopropanol. Ann. NY Acad. Sci. 381: 344-349.

Epstein, S.S., E. Arnold, J. Andrea, W. Bass and Y. Bishop. 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23: 288-325.

Ericsson, R.J. 1970. Male antifertility compounds: U-5899 as a rat chemosterilant. J. Reprod. Fert. 22: 213-222.

Federal Register. 1980. Guidelines and methodology used in the preparation of health effects assessment chapters of the consent decree water quality criteria. 45: 79347-79357.

Fedyanina, V.N. 1968. Sanitary-toxicologic study of epichlorohydrin in order to set standards for its content in bodies of water. Gig. i Sanit. 33(1): 46-51

Fishbein, L. 1976. Industrial mutagens and potential mutagens. I. Halogenated aliphatic derivatives. Mutat. Res. 32(3/4): 267-307.

Fitzpatrick, R.W., H. Jackson and N.A. Dickinson. 1978. Effect of racemic and S(+)γ-chlorohydrin-1-phosphate on glyceraldehyde-3-phosphate dehydrogenase in relation to its contraceptive action. Contraception. 18: 477.

Fjellstedt, T.A., R.H. Allen, B.K. Duncan and W.B. Jakoby. 1973. Enzymatic conjugation of epoxides with glutathione. J. Biol. Chem. 248: 3702-3707.

Fomin, A.P. 1966. Biological effect of epichlorohydrin and its hygienic significance as an atmospheric pollutant. Gig. Hyg. Sanit. 31: 357-363.

Fomin, A.P. 1968. Effect of low concentrations of epichlorohydrin vapor on the animal organism. Vap. Gig. Atmos. Vozdukha Planirovki Naselen. Mest. (Moskva). p. 50-56.

Freuder, E. and C.D. Leake. 1941. The toxicity of epichlorohydrin. Berkeley, CA. Univ. Cal. Publ. Pharm. 2(5): 69-78.

Gage, J.C. 1959. The toxicity of epichlorohydrin vapour. Br. J. Ind. Med. 16: 11-14.

Grigorowa, R., Gohlke, R., R. Roghe and H.J. Weigmann. 1977. MAC value for epichlorohydrin at normal and elevated ambient temperatures. Z. Gesamte Hyp. Ihre Grenzgeb. 23(9): 620-623.

Hahn, J.D. 1970. Post-testicular antifertility effects of epichlorohydrin and 2,3-epoxypropanol. Nature (London). 226: 87.

Hansch, C. and A.J. Leo. 1979. Substitute Constants for Correlation Analysis in Chemistry and Biology. John Wiley and Sons, NY.

Hayakawa, T., S. Udenfriend, H. Yagi and D.M. Jerina. 1975. Substracts and inhibitors of hepatic glutathione-S-epoxide transferase. Arch. Biochem. Biophys. 170: 438-451.

Hemminki, K. 1979. Fluorescence study of DNA alkylation by epoxides. Chem. Biol. Interact. 28(2-3): 269-278.

Hemminki, K., J. Paasivirta, T. Kurkirinne and L. Virkki. 1980. Alkylation products of DNA bases by simple epoxides. Chem-Biol. Interact. 30(3): 259-270.

IARC (International Agency for Research on Cancer). 1982. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Chemical Industrial Processes and Industries Associated with Cancer in Humans. Suppl. 4. p. 122-123.

Ippen, H. and V. Mathies. 1970. Die protrahierte Veratzung (unterbesonderer Berucksichtigung der Hautscha der durch Epoxide und Propansulton). Herufsdermatosen. 18: 144-165. (Ger.)

John, J.A., J.F. Quast, F.J. Murray, et al. 1979. The effects of inhaled epichlorohydrin on the semen of rabbits and on the fertility of male and female rats. Unpublished report, Toxicol. Res. Lab., Health and Environ. Sci., Dow Chemical U.S.A., Midland, MI.

John, J.A., J.F. Quast, F.J. Murray, L.S. Calhoun and R.E. Staples. 1983. Inhalation toxicity of epichlorohydrin: Effects on fertility in rats and rabbits. Toxicol. Appl. Pharmacol. 68: 415-423.

Jones, A.R. and G. Fakhouri. 1979. Epoxides as obligatory intermediates in the metabolism of γ-halohydrins. Xenobiotica. 9: 595-599.

Jones, A.R. and R.W. O'Brien. 1980. Metabolism of three active analogues of the male antifertility agent alpha-chlorohydrin in the rat. Xenobiotica. 10(5): 356-370.

Jones, A.R., P. Davies, K. Edwards and H. Jackson. 1969. Antifertility effects and metabolism of alpha- and epichlorohydrins in the rat. Nature (London). 224(5214): 83.

Jones, A.R., D.H. Milton and C. Murcott. 1978. The oxidative metabolism of γ-chlorohydrin in the male rat and the formation of spermatocoeles. Xenobiotica. 8: 573.

Jones, A.R., P.M. Mashford and C. Murcott. 1979a. The metabolism of 3-amino-1-chloropropan-2-ol in relation to its antifertility activity in male rats. Xenobiotica. 9: 253-261.

Jones, A.R., G. Fakhouri and P. Gadiel. 1979b. The metabolism of the soil fumigant 1,2-dibromo-3-chloropropane in the rat. Experientia. 35: 1432-1434.

Kalla, N.R. and M.P. Bansal. 1977. In vivo and in vitro alkylation of testicular cysteine by alpha-chlorohydrin. Ind. J. Exp. Biol. 15: 232-233.

Kanada, T. and M. Uyeta. 1978. Mutagenicity screening of organic solvents in microbial systems. Mutat. Res. 54: 215.

Kawabata, A. 1981. Studies on the carcinogenic activity of epichlorohydrin by oral administration in male Wistar rats. J. Nara Med. Assoc. 32: 270-280.

Kilian, D.J., T.G. Pullin, T.H. Conner, M.S. Legator and H.N. Edwards. 1978. Mutagenicity of epichlorohydrin in the bacterial assay system: Evaluation by direct _in vitro_ activity and _in vivo_ activity of urine from exposed humans and mice. Mutat. Res. 53: 72.

Kirkhart, B. 1981. Micronucleus test on 21 compounds. _In_: Evaluation of Short-Term Tests for Carcinogens, F.J. deSerres and J. Ashby, Ed. Elsevier/North Holland, Amsterdam. p. 698-704.

Knapp, A.G.A.C., C.E. Voodg and P.G.N. Kramers. 1982. Comparison of the mutagenic potency of 2-chloroethanol, 2-bromoethanol, 1,2-epoxybutane epichlorohydrin and glycidaldehyde in _Klebsiella pneumoniae_, _Drosophila melanogaster_ and L5178Y mouse lymphoma cells. Mutat. Res. 101: 199-208.

Kolmark, G. and N.H. Giles. 1955. Comparative studies of monoepoxides as inducers of reverse mutations in Neurospora. Genetics. 40: 890-902.

Konishi, T., A. Kawabata, A. Denda, et al. 1980. Forestomach tumors induced by orally administered epichlorohydrin in male Wistar rats. Gann. 71: 922-923.

Kremneva, S.N. 1960. Investigation of toxicity of epichlorohydrin and dichlorohydrin. Pr. Toksikol. Moscow Sb. 123-131.

Kremneva, S.N. and M.S. Tolgskaya. 1961. Toxicology of epichlorohydrin. Toksikol. Nov. Prom. Khim. Veschestv. 2: 28-41.

Kucerova, M., V.S. Zhurkov, Z. Polwkova and J.E. Ivanova. 1977. Mutagenic effect of epichlorohydrin. II. Analysis of chromosomal aberrations in lymphocytes of persons occupationally exposed to epichlorohydrin. Mutat. Res. 48: 355-360.

Laskin, S., A.R. Sellakumar, M. Kuschner, et al. 1980. Inhalation carcinogenicity of epichlorohydrin in noninbred Sprague-Dawley rats. J. Natl. Cancer Inst. 65(4): 751-758.

Lawrence, W.H., M. Malik, J.E. Turner and J. Autain. 1972. Toxicity profile of epichlorohydrin. J. Pharm. Sci. 61(11): 1712-1717.

Lawrence, W.H., M. Malik and J. Autain. 1974. Development of a toxicity evaluation program for dental materials and products. II. Screening for systemic toxicity. J. Biomed. Mater. Res. 8: 11-34.

Mabey, W. and T. Mill. 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions. J. Phys. Chem. Ref. Data. 7: 383-415.

Marks, T.A., F.S. Gerling and R.E. Staples. 1982. Teratogenic evaluation of epichlorohydrin in the mouse and rat and glycidol in the mouse. J. Toxicol. Environ. Health. 9: 87-96.

Mashford, P.M. and A.R. Jones. 1978. The antifertility action of γ-chlorohydrin: Enzyme inhibition by γ-chlorohydrin phosphate. Experientia. 34: 1267.

Matsui, S., T. Murakami, T. Sasaki, Y. Hirose and Y. Iguma. 1975. Activated sludge degradability of organic substances in the waste water of the kashima petroleum and petrochemical industrial complex in Japan. Prog. Water Technol. 7: 645-59.

McMahon, R.E., J.C. Cline and C.Z. Thompson. 1979. Assay of 855 test chemicals in ten testes strains using a new modification of the Ames test for bacterial mutagens. Cancer Res. 39: 682-693.

Migliore, L., A.M. Rossi and N. Loprieno. 1982. Mutagenic action of structurally related alkene oxides on Schizosaccharomyces pombe: The influence in vitro of mouse-liver metabolizing system. Mutat. Res. 102: 425-437.

Milby, T.H., M.D. Whorton, H.A. Stubbs, C.E. Ross, R.E. Joyner and L.I. Lipshultz. 1981. Testicular function among epichlorohydrin workers. Br. J. Ind. Med. 38(4): 372-377.

Mohn, G., P. Kerklaan, P. deKnijff and S. Bouter. 1981. Influence of phenotypic expression lag and division delay on apparent frequencies of induced mutations in Escherichia coli K12. Mutat. Res. 91: 419-425.

Mohri, H., D. Suter, P.D.C. Brown-Woodman, I.G. White and D.D. Ridley. 1975. Identification of the biochemical lesion produced by γ-chlorohydrin in spermatozoa. Nature. 255-275.

Moore-Brown, M.M. and D. Clive. 1979. The L5178Y/TK$^{+/-}$ mutagen assay system: In situ results. Banbury Report. 2: 71-88.

Mukai, F.H. and I. Hawryluk. 1973. The mutagenicity of some halo-ethers and halo-ketones. Mutat. Res. 21: 52-53.

Neely, W.B. 1976. Predicting the flux of organics across the air/water interface. Control Hazard Mater. Spills, Proc. Natl. Conf. Third. p. 197-200.

NAS (National Academy of Science). 1980. Drinking Water and Health, Vol. III. National Academy Press, Washington, DC. p. 111-124.

Norppa, H., K. Hemminki, M. Sorsa and H. Vainio. 1981. Effect of monosubstituted epoxides on chromosome aberrations and SCE in cultured human lymphocytes. Mutat. Res. 91: 423-250.

Pallade, S., M. Dorobantu, G. Rotaru and E. Garbielescu. 1967. Experimental studies of intoxication by epichlorohydrin. Arch. Mel. Prof. 28: 505-516. (Fre.)

Picciano, D. 1979a. Cytogenic investigation of occupational exposure to epichlorohydrin. Mutat. Res. 66: 169-173.

Picciano, D. 1979b. Faulty experimental design and underutilization of cytogenetic data. Benzene and epichlorohydrin. Ann. NY Acad. Sci. 329: 321-327.

Pilny, M.K., T.S. Lederer, J.S. Murray, et al. 1979. Epichlorohydrin-subchronic studies. IV. The effects of maternally inhaled epichlorohydrin on rat and rabbit embryonal and fetal development. Unpublished report, Toxicol. Res. Lab., Health Environ. Sci., Dow Chemical U.S.A., Midland, MI.

Quast, J.F., J.W. Henck, B.J. Postma, D.J. Scheutz and M.J. McKenna. 1979a. Epichlorohydrin subchronic studies. I. A 90-day inhalation study in laboratory rodents (Fischer 344 rats, Sprague-Dawley rats and $B6C3F_1$ mice). Dow Chemical U.S.A., Midland, MI. 166 p.

Quast, J.F., T.S. Lederer, B.J. Postma, D.J. Scheutz, J.A. John and M.J. McKenna. 1979b. Epichlorohydrin -- Subchronic studies. II. A 12-day inhalation study in laboratory rodents (Fischer 34 rats, Sprague-Dawley rats and $B6C3F_1$ mice). Dow Chemical U.S.A., Midland, MI. 57 p.

Rapoport, I.A. 1948. The effect of ethylene oxide, glycide and glycol on genetic mutations. Doklady Akademii Nauk SSR. 60: 469-472. (Russian with English translation)

Riesser, G.H. 1979. Chlorohydrines. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 5, M. Grayson and D. Eckroth, Ed. John Wiley and Sons, Inc., NY. p. 858-864.

Rossi, A.M., D. Migliore, D. Lascialfari, I. Sbrana and N. Loprieno. 1983a. Genotoxicity, metabolism, and blood kinetics of epichlorohydrin in mice. Mutat. Res. 118: 213-226.

Rossi, A.M., L. Migliore, N. Loprieno, M. Romano and M. Salmona. 1983b. Evaluation of epichlorohydrin (ECH) genotoxicity. Microsomal epoxide hydrolase-dependent deactivation of ECH mutagenicity in Schizosaccharomyces pombe in vitro. Mutat. Res. 109: 41-52.

Rotaru, G. and S. Pallade. 1966. Experimental studies of histopathological features in acute epichlorohydrin toxicity. Morfol. Norm. Patol. 11: 155-164.

Sasaki, S. 1978. The scientific aspects of the chemical substance control law in Japan. In: Aquatic Pollutants: Transformation and Biological Effects, O. Hutzinger, L.H. Von Letyoeld and B.J.C. Zoeteman, Ed. Pergamon Press, Oxford. p. 283-98.

Schultz, V.C. 1964. Fettelber und chronisch-asthmoide Bronchitis nach Inhalation eines Farbenlosungsmittels (Epichlorohydrin). Dtsch. Med. Wschr. 89: 1342-1344. (Ger.)

Shumskaya, N.I. and N.M. Karamzina. 1966. An evaluation of the functional state of rat kidneys during poisoning by industrial substances. Toksikol. Nov. Prom. Khun. Veshchestv. 8: 14-27.

Smith, F.A., P.W. Langvardt and J.D. Young. 1979. Pharmacokinetics of epichlorohydrin (EPI) administered to rats by gavage or inhalation. Dow Chemical U.S.A., Toxicology Research Laboratory, Midland, MI. 52 p.

Smyth, H.F., Jr. and C.P. Carpenter. 1948. Further experience with the range finding test in the industrial toxicology laboratory. J. Ind. Hyg. Toxicol. 30(1): 63-68.

Smyth, H.F., Jr., C.P. Carpenter, C.S. Weil, U.C. Pozzani and J.A. Streigel. 1962. Range-finding toxicity data, List VI. Ind. Hyg. J. 23: 95-96.

Sram, R.J. 1980. Cytogenetic analysis of peripheral lymphocytes as a method for monitoring environmental levels of mutagens. Ind. Environ. Xenobiotics, Proc. Int. Conf. p. 187-194.

Sram, R.J., M. Cerna and M. Kucerova. 1976. The genetic risk of epichlorohydrin as related to the occupational exposure. Biol. Zbl. 95: 451-462.

Sram, R.J., Z. Zudova and N.P. Kuleshov. 1980. Cytogenetic analysis of peripheral lymphocytes in workers occupationally exposed to epichlorohydrin. Mut. Res. 70: 115-120.

Sram, R.J., L. Tomatis, J. Clemmessen and B.A. Bridges. 1981. An evaluation of the genetic toxicity of epichlorohydrin. Mutat. Res. 87: 299-319.

Strauss, B. and S. Okubo. 1960. Protein synthesis and the induction of mutagens in Escherichia coli by alkylating agents. J. Bacteriology. 79: 464-473.

Thorgeirsson, A. and S. Fregert. 1977. Allergenicity of epoxy resins in the guinea pig. Acta Dermatovener (Stockholm). 57: 253-256.

Tsuchimoto, T. and B.E. Matter. 1981. Activity of coded compounds in the micronucleus test. In: Evaluation of Short-Term Tests for Carcinogens, F.J. Seeres and J. Ashby, Ed. Elsevier/North Holland, Amsterdam. p. 705-711.

Turner, M.A. 1971. Effects of α-chlorhydrin upon the fertility of spermatozoa of the cauda epididymidis of the rat. J. Reprod. Fert. 24: 267-269.

U.S. EPA. 1983. Draft. Health Assessment Document for Epichlorohydrin. Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA. 1984. Final Health Assessment Document for Epichlorohydrin. Office of Health and Environmental Assessment, Washington, DC. EPA 600/8-83-032 F.

Van Duuren, B.L., C. Katz and B.M. Goldschmidt. 1972. Direct-acting alkylating carcinogens -- Chloro ethers and related compounds. Toxicol. Appl. Pharmacol. 22: 279-280.

Van Duuren, B.L., B.M. Goldschmidt, C. Katz, I. Seidman and J.S. Paul. 1974. Carcinogenic activity of alkylating agents. J. Natl. Cancer Inst. 53: 695-700.

Van Esch, G.J. 1981. Induction of Preneoplastic Lesions in the Forestomach of Rats After Oral Administration of 1-Chloro-2,3-Epoxypropane. I. Range Finding Studies. Prepared by Ryksinsitute Voor De Volksgezondheld Bilthoven Rapport nr. 627805 005.

Van Esch, G.J. 1982. Induction of Preneoplastic Lesions in the Forestomach of Rats After Oral Administration of 1-Chloro-2,3-Epoxypropane. II. Carcinogenicity Study. Prepared by Ryksinstitute Voor De Volksgezondheld Bilthoven Rapport nr. 627805 005.

Venable, J.R., C.D. McClimans, R.E. Flake and D.B. Dimick. 1980. A fertility study of male employees engaged in the manufacture of glycerine. J. Occup. Med. 22(2): 87-91.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY. p. 611-613.

Vogel, E.A., A. Schalet, W.R. Lee and F. Wurgler. 1981. Mutagenicity of selected chemicals in Drosophila. In: Comparative Chemical Mutagenesis, J.F. deSerres, Ed. Environ. Sci. Res. Ser. Vol. 24, Chap. 9. Plenum Publisher.

Voogd, C.E. 1973. Mutagenic action of epoxy compounds and several alcohols. Mutat. Res. 21: 52-53.

Voogd, C.E., J.J. van der Stel and J.J.J.A.A. Jacobs. 1981. The mutagenic action of aliphatic epoxides. Mutat. Res. 89: 269-282.

Wade, D.R., S.C. Airy and J.E. Sinsheimer. 1978. Mutagenicity of aliphatic epoxides. Mutat. Res. 58: 217-223.

Weigel, W.W., H.B. Plotnick and W.L. Conner. 1978. Tissue distribution and excretion of ^{14}C-epichlorohydrin in male and female rats. Res. Commun. Chem. Pathol. Pharmacol. 20(2): 275-288.

Weil, C.S., N. Condra, C. Hann and J.A. Streigel. 1963. Experimental carcinogenicity and acute toxicity of representative epoxides. Am. Inc. Hyg. Assoc. J. 24: 305-325.

White, A.D. 1980. *In vitro* induction of sister chromatid exchange in human lymphocytes by epichlorohydrin with and without metabolic activation. Mutat. Res. 78: 171-176.

Whitman, R.G. 1923. A preliminary experimental confirmation of the two-film theory of gas absorption. Chem. Metallurg Eng. 29: 146-148. (See also Burns et al., 1981)

Wurgler, F.E. and U. Graf. 1981. Mutagenic activity of ten coded compounds in the *Drosophila* sex-linked recessive lethal assay. *In*: Evaluation of Short-Term Tests for Carcinogens, F.J. de Serres, Ed. Elsevier/North Holland, Amsterdam. p. 666-672.